COLLECTED PAPERS

from

THE MEDICAL RESEARCH LABORATORIES PARKE, DAVIS & CO. DETROIT, MICH.

DR. E. M. HOUGHTON, Director.

Reprints—Vol. 8

1919—1920

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 194, 1919.

[Reprinted from the *Journal of the American Pharmaceutical Association*,
Vol. VIII, No. 1. January, 1919.]

PHARMACOLOGICAL ASSAYING—HISTORICAL AND DESCRIPTIVE.*

By Herbert C. Hamilton.

(From the Medical Research Laboratories, Parke, Davis & Co., Detroit, Mich.)

The first discovery of the value of medicinal substances and their later development was based very largely on pharmacologic observations. During more recent times this has been looked upon as almost the sole means for a rational selection of remedies and for the establishing of correct dosage.

It is only of comparatively recent years, however, that pharmacology has been recognized scientifically as a method of ascertaining the value of a medicinal preparation. For the most part if no chemical method existed for standardizing, entire dependence was placed on the standard methods for extraction and on certain physical tests. Later, when it was recognized that a worthless sample of a medicinal drug would make an extract not differing in any apparent respect from one from an active sample, it was very evident that an assay process was a necessity.

Pharmacologic assaying cannot be applied to any drug which induces no typical reaction when administered to an animal or applied to living tissue, and it is unnecessary to apply it to drugs possessing an active constituent with well marked chemical characteristics. In general, the attitude on this subject is that whenever possible pharmacologic assaying is adapted for such drugs as are not amenable to chemical assay.

On the other hand, there is the extremist, who voices the opinion of not a few when he says that every medicinal preparation amenable to a pharmacologic test should be standardized. This, however, is scarcely a logical viewpoint.

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*Presented before Section on Historical Pharmacy, A. Ph. A., Chicago meeting, 1918.

The objection voiced against biological standardization is not against the method as a general proposition, but largely against the method in its particular application—that it is qualitative only. There is no question anywhere of the fact that only by animal or human experimentation can the properties of a drug be established. The question is whether the test can be made quantitative and the value of the substance be measured to establish the dosage. It is only of recent years that the therapeutic properties could even occasionally be assumed from the chemical composition. We are still to a certain extent dependent on the natives to suggest the importance of a drug, by the use they make of it—a use based on a more or less accidental observation of its effects on themselves or animals.

As illustration of these points it may be noted that hellebore was discovered to have medicinal properties by Melampe, a shepherd who traced the diarrhea in his sheep to their having eaten of this plant.

Acetanilid was by accident found to have antipyretic properties by being given to one of Prof. Kussmanka's assistants, causing an alarming lowered temperature.

Many drugs, such as quinine and cocaine, were used by the natives medicinally with no record of their first discovery. In some cases human use followed the observation that animals apparently chose certain plants for relief from injury or disease. These records are, however, not very trustworthy.

Confining this historical account to drugs and medicinal substances now commonly standardized by use of animals eliminates much of interest in the history of the materia medica, but leaves for consideration some of the most important medicinal substances, *Cannabis Sativa,* Ergot, the heart tonics of the Digitalis series, and the Suprarenal and Pituitary gland extracts.

The writer may be pardoned if he draws somewhat from his own experience, for 19 years of close acquaintance with the actual standardization of the drugs mentioned covers the greatest part of the period during which such standardization in its restricted sense has been practiced.

CANNABIS SATIVA.

Cannabis Sativa, or, when grown in India, designated as *Cannabis Indica*, was known and used 1000 years B. C. It may have been the substance referred to under different names, as for example Nepenthe. Its effects are very wonderfully described in Dumas' "Count of Monte Cristo."

Cannabis Sativa was probably never standardized with any degree of accuracy by the use of any other animal than the dog. Fraenkel[1] confirms the experience of most investigators in stating that rabbits are immune to its action. Guinea pigs are also practically without reaction to this drug in any reasonable quantity. Cats are susceptible but are unsatisfactory test animals in many respects.

My personal experience with its physiological assay on dogs began in 1899, but this was merely to continue a practice which had obtained since 1894-5. Houghton,[2] in 1897, read a paper on Physiological Standardization, in which he referred to its use in establishing the reliability of cannabis preparations, but at that time gave no details of the method applied. Twenty-seven samples were assayed, only thirteen of which proved to be active when administered to animals.

One of the first authors to mention the use of dogs and to describe specifically the effect of the drug is Ponthieu[3], in 1901, who says: "To verify the action of Cannabis Indica the dog is used, and the drug is administered in the form of an extract; its physiological action manifests itself later in a vacillating gait, ataxia, depression of temperature, and finally complete insensibility."[4]

While no accurate description of the assay method originated by Houghton, and regularly practiced since, appeared until 1908, a paper by Thomas Maben[5] was read before the Dundee meeting of the British Pharmaceutical Conference in 1902, on the physiological action of *Cannabis Indica*—a paper "based on observations communicated to him in the course of a discussion with H. C. Hamilton," quoted from Proc. A. Ph. A., 1903, page 804.

Famulener and Lyons[6] have recorded the first accurate description of the physiological assay of *Cannabis Indica,* including doses of official preparations, characteristic effects, and the end-point to be observed in establishing the value of the drug.

Fraenkel,[1] in an article published the same year, described the action of the drug on dogs, but gave meager data on dosage, as the samples tested were cannabinol and its derivatives. The work there recorded was qualitative only.

The details of the method in practice at the time of my first acquaintance with the work were identical with those described by Famulener and Lyons with the exception that in addition to recording the degree of incoördination, other symptoms, such as degree of preliminary excitement, of drowsiness, and of fall in temperature, were preserved as part of the record. At some time in 1900 record of these by-effects was discontinued as being non-essential and subject to greater individual variation than the degree of incoördination which is typical of cannabis intoxication. In reporting on the pharmacological identity of American and Indian cannabis Houghton and Hamilton[7] described the method as modified and regularly applied at that time.

While practically all the writers up to this time had selected the dog as the test animal, Goodall[8] writes, "At present my standard is that a dose of 1/4 grain should kill or deeply narcotize frogs of 20 Gm."

Haskell[9] refers to Houghton's as the only assay method known.

This method is again described in concise form in the Report of the A. Ph. A. Committee on Physiological Testing.[10]

Pittenger,[11] in 1914, published the same assay method, but with no dosage specified and no material changes.

Pearson[12] emphasizes the need of and difficulty in selecting susceptible dogs, also noting that continued dosing does not produce any immunity.

Eckler and Miller[13] seem to be the first to describe the use of a particular breed of dog but not in the sense of specifying the exclusive use of this breed. Hamilton, Lescohier, and Perkins[14] touched on a phase of cannabis standardization not apparently considered by other investigators. In order to corroborate for human therapy the fact established by animal experimentation that cannabis preparations are equally valuable from whatever source the crude drug is derived, and require only the ordinary physiological assay, these investigators carried out several series of tests on themselves, using both Indian and American grown drug. Their conclusion is that no difference in the effects of the two varieties could be detected.

Finally, we come to the U. S. P. Revision Committee's Report now embodied in U. S. P. IX[15] and made official for the assay of *Cannabis Sativa*. This report includes several steps not previously suggested as essential in assaying this drug, namely, 1st, Fox terriers as the test animal, but not exclusively; 2nd, Doses of 0.03 cc for F. E. Cannabis, 0.3 cc for the tincture, 0.004 gm. for the extract; 3rd, Preliminary fast of 24 hours for the dogs used.

Previous to this the only requirement in the test animal was susceptibility. The doses suggested by Houghton and Hamilton, Famulener and Lyons, and Eckler and Miller were 0.01 gm. of extract, 0.1 cc of fluid extract, and 1.0 cc of tincture per kilo of dog weight. The period of fasting was suggested at several intervals up to 12 hours. The intention of the official method is to require these preparations to produce an observable reaction with the specified doses, while previous authors made use of doses of such size that a weaker preparation would have a measurable reaction, evident but less intense than that required for a standard preparation. This feature shortens the test of a weak preparation in that a clue to its activity is likely to be obtained in the first test, while by the official method only a standard (or better) preparation would show an effect.

It is evident, therefore, that the official method differs only in some of its details from that previously followed, but that these complicate the method by including non-essential details and by increasing the difficulties of the test.

ERGOT.

In very early times this drug was used in obstetrics by the Chinese and the Romans. Salerne,[1] in 1754, and Tessier,[2] in 1778, found that gangrene occurs in young pigs after administration of ergot.

Dietz noted that one to three ounces of ergot would cause gangrene of the combs of birds.

Wiggers[3] fed 9 grains of an extract he called "ergotin," obtained by alcoholic extraction, to a cock and caused convulsions and death. This probably occurred too quickly for the typical

bluing of the comb to appear, since he noted only that the comb became cold.

Bonjean[4] obtained an aqueous extract purified by precipitation with alcohol to which he also gave the name "ergotine." This caused the typical bluing of comb and wattles and a narcotic condition, which demonstrated to him that it contained the therapeutic agent.

Kobert,[5] in carrying out investigations of ergot bodies in 1884, used all the laboratory animals, including cocks, frogs, pigs, rabbits, cats, and dogs. He used also the isolated uterus of the sheep and considered this the most suitable method of testing ergot, but as a second and final test it must produce abortion in pregnant animals with no other untoward effect.

Jacobi's work in 1897[6] was probably the most important to that date because he carefully checked up his chemical investigations by means of physiological tests. He noted the action of ergot on the uterus, on the cock's comb, and on blood pressure, the three characteristic effects of this drug.

All the work recorded to this time had been on experimental bodies, with no reference to standardization of commercial products. Houghton,[7] in 1898, proposed, in a paper before this Association (the A. Ph. A.), applying the Cock's Comb Method for the routine assay of commercial ergot preparations, the method of administration then followed being that of feeding the crude drug and by means of a catheter introducing fluid preparations into the rooster's crop.

About this time the work of Barger, Carr and Dale,[8] who wrote voluminously at this period, seemed to have cleared up much of the uncertainty regarding the identity and character of the active constituents of ergot. They showed that different constituents were responsible for the different physiological effects noted. Thus they demonstrated that the aqueous extract as well as an alcoholic can contain an active agent. Ergotoxine, an alkaloid, appears to be the agent causing the bluing of the cock's comb; *p*-oxyphenylethylamine or tyramine is the pressor agent, although the alkaloid acts in this way, too; while *B*-iminazoylethylamine or histamine is the principle which acts on the non-pregnant uterus and in most cases lowers the blood pressure of anesthetized dogs and rabbits. This work shows conclusively why

no assay method based on the amount of any one active constituent present is adapted to the standardization of this drug.

Dohme and Crawford,[9] after considerable experimentation, evolved the method of injecting hypodermically solutions of the fluidextract and of Keller's Cornutine, using 5 cc of the former and equivalent amounts of the latter. They concluded that cornutine represents practically all of the therapeutically active substances of ergot and that an assay for Keller's cornutine is the correct means of standardizing this drug for its vaso-constrictor virtues. Dohme[10] later concluded that this was not a correct method because samples of ergot very low in cornutine were quite active when tested by physiological means.

Barger and Dale[11] suggested a method known as the vasomotor reversal. The end-point of this reaction is the complete neutralization of the pressor effect of 0.1 mg. of adrenalin.

The later recognition of the various active agents of ergot easily explains why this method is very inaccurate.

Kehrer[12] found the isolated cat uterus suspended in Ringer's fluid the most satisfactory method for assay, and believed that the manufacturer had in that a means of assuring standardized products. From his work with this and with the cock's comb method he concluded that the same active agent is not concerned in the two effects, since one disappeared more rapidly than the other.

Goodall[13] agreed with Kehrer in the use of cats, but employed as the end-point the action on blood pressure—20 minims of liquid extract intravenously administered should raise the pressure 20 mm. of mercury in an animal of 1500 Gm. He, however, recognized the presence of both pressor and depressor substances, and concluded that "In the present state of knowledge it is hardly possible to adjust the therapeutic dosage of ergot to physiological findings."

Cronyn and Henderson[14] pointed out that failure to obtain satisfactory blood pressure records is largely due to the anesthetic —a volatile anesthetic partially nullifying the pressor action. This has also been observed by others. They reached the same conclusion as that of Goodall, that no thoroughly reliable method was known for establishing uniformity in ergot extracts. Their work with the cock's comb method is, however, open to criticism

on account of using different breeds of roosters and not more carefully standardizing the technic.

Probably no more extensive experiments have been carried out to select a satisfactory assay method for ergot than were those of Edmunds and Hale,[15] who reviewed the literature critically, examined the various methods which had been suggested, and finally selected as least subject to inaccuracies the cock's comb method, the end-point recommended being that 1 cc of the fluidextract injected deeply into the breast muscles must blacken the comb in one hour. As a standard they suggest that 1 cc of fluidextract blacken the comb to the same extent as 2½ mg. ergotoxin phosphate.

Pittenger and Vanderkleed,[16] in 1914, suggested as the most logical and accurate method for standardizing ergot, its action on the isolated uterus muscle. This method, in the light of the chemical and pharmacological investigations of Barger and Dale, is not applicable for their standardization. They consider ergotoxin to be the most valuable principle in ergot and also that it has little action on the uterus.

My personal experience with the standardization of ergot suggests the use of White Leghorn cocks not over 1 year old, sufficiently susceptible to the action of ergot so that 1 cc of the fluidextract will blacken the comb in a typical manner and to a reasonable degree in one hour. The roosters for test purposes have an individual record of the average degree of blackening to be expected and are frequently retested with a standard fluidextract to verify their susceptibilities.

Knowing as we do that different constituents of ergot have different pharmacologic effects, it is difficult to select for test purposes a reaction which surely represents the desirable therapeutic action of the drug.

The chemist finds certain constituents to be present; the pharmacologist determines their typical effects; but rarely has the physician had full opportunity to conclude as to which is the substance concerned in the therapeutic effects of the drug.

As an oxytocic agent one would naturally believe that only the constituent acting on the uterine muscle is of value, while as a hemostatic the pressor effect seems the logical measure.

But histamine, which has a selective action on the uterine

muscle, has not proved to be a valuable oxytocic agent when used alone. Further, it lowers the blood pressure of most anesthetized animals and thus obscures the pressor effect [of the ergot] and makes the blood pressure test of the drug an uncertain measure of its value as a hemostatic.

Tyramine, another of the constituents isolated from ergot, is said to act on the uterus and to raise the blood pressure, both effects probably due to its action on unstriped muscular tissue.

Ergotoxin has much the same therapeutic effect, and pharmacologically has the typical action of bluing the cock's comb.

The logical conclusion, therefore, from this is that the cock's comb reaction is not obscured by counteracting substances, as is the pressor test, and with our present knowledge is the most satisfactory of the various tests proposed.

THE DIGITALIS SERIES OF HEART TONICS.

The earliest recorded attempts actually to standardize the members of this series are apparently those of Fagge and Stevenson[1] in 1866. They claimed that the physiological tests of these medicinal substances would be of great medicolegal importance.

They carried out tests on most of the members of the series but particularly on digitalin. The method used was that later called the Focke Method and consisted in exposing the heart of the weighed frog, which was then attached to a cork. The solution was injected subcutaneously into the thighs and the time noted when the heart stopped in systole. The time elapsing between injection and systolic stoppage was selected as the basis for the relative toxicities of the different samples. They considered frogs much more satisfactory than the higher animals because of ease in examining the different organs and the rapidity of their reaction to the drug.

Koppe[2] carried out similar experiments on the separated constituents of digitalis, using the same technic and end-point as the earlier writers. He used other animals also, dogs, cats and rabbits, to which the drug was administered mostly subcutaneously, noting in the dog and rabbit the changes in the rate and strength of the heart beat, in the cat and dog also the amount necessary to induce vomiting.

In 1881, Bennefield[3] undertook the examination of tinctures

of digitalis from different parts of Germany. Chemical and physical methods failing, he applied physiological tests, using the rabbit. His technic was that later adopted by Hatcher for his cat method, namely, to inject the solution slowly into the jugular vein until the animal dies. The solution injected was prepared by evaporating the tincture to constant weight and treating the residue with water at a moderate temperature, using a filtered solution for injection.

Fraenkel,[4] in the same year, examined different preparations of digitalis on the dog. The animal was curarized and injected subcutaneously. He observed three typical effects of digitalis, the increased blood pressure, decreased rate and increased amplitude of the heart beat.

Gley,[5] in 1888, tested ouabain and strophanthin on the laid-bare frog's heart and observed that with equal doses systolic stoppage was accomplished in half the time with the former. He also determined their toxicity on guinea pigs, dogs, and rabbits.

Reusing[6] compared the action of strophanthus and digitalis, using frogs. He not only studied the effect on the exposed heart, as in previous methods, but applied also a perfusion method.

Bardet[7] examined the various active constituents of digitalis, employing frogs and rabbits in the method known as the M. L. D. method, the first recorded account of the use of frogs in this the simplest application of the physiological test, the method adopted by Houghton for quantitative assay purposes.

Fouquet[8] also determined only the M. L. D., using frogs, dogs and rabbits, administering the material subcutaneously with as little alcohol as would dissolve the substance.

Prevost,[9] examining Swiss Pharmacopoeial digitalis extracts, used frogs and chose for the end-point the minimum systolic dose. He considered the frog by far the best adapted to the work.

Houghton,[10] in 1898, in October took the first step in advance of other pharmacologists of this time by adopting as a routine procedure the physiologic assay of the heart tonics on frogs, using the M. L. D. as the end-point. This method was further elaborated and presented before the Pharmaceutical Section of the 7th International Congress of Applied Chemistry on May 31,

1909, at which time he suggested a Heart Tonic Unit based on the M. L. D. and proposed this as an international standard for the assay of the digitalis series.

Jacquet,[11] recognizing the importance and the applicability of the physiological test in arriving at exact dosage of digitalis, described his method and recorded his results in 1897, but did not propose the routine application of the method until December, 1898. His method was the systolic stoppage of the frog's heart, but he used rabbits as well, adopting as an end-point the minimum lethal dose.

Fränkel,[12] taking up the subject again, used the systolic stoppage of the frog's heart as the end-point but made an important forward step in fixing the time at which stoppage in systole must take place as one hour, thus varying the dose instead of the time. This approaches still more closely the method of Focke and Gottlieb, and is almost identical with the present U. S. P. method.

Famulener and Lyons[13] adopted the same general method, probably about the same time, in studying the relative values of different digitalis extracts and the active constituents of digitalis.

Ziegenbein,[14] using a method credited to Hans and Arthur Meyer but really originated by Fagge and Stevenson, examined a number of different species of digitalis and found them to differ greatly in toxicity. He selected a two-hour interval in which the drug should act to induce the systolic stoppage.

With all the imperfections which he had observed in the physiological methods he considered that only by the use of the frog could one obtain a degree of uniformity in the activity of digitalis preparations.

Moschkowitsch,[15] in 1903, on the basis of considerable experimentation, regretted to report that he failed to substantiate the results of Focke, Prevost, and others by use of frogs, but his failure seemed to carry no particular weight against physiological testing.

The reason for this is probably twofold: first, because some of his work is open to criticism; and, second, because all who have experimented in pharmacologic assaying recognize the difficulties and discouragements involved, and, further, realize that chemical methods have even less evidence in their favor.

Focke[16] wrote voluminously on the physiological assay of

digitalis and a method known by his name was given tangible form in 1902.

He applied the principle first used by Fagge and Stevenson, but modified for quantitative results. He obtains a value $V = p/dt$, in which p is weight of the frog, d the dose, and t the time of systolic standstill.

The objections to this, the second modification of the frog-heart method in its quantitative application, are first, that the short time selected for obtaining results, namely, 7 to 10 minutes, is too short for complete absorption of the drug and is thus inapplicable to extracts containing much inert material; second, the laying bare of the heart of an unanesthetized animal is contrary to the best pharmacologic procedure, and, further, is certainly a factor in affecting the results adversely; third, the use of so few frogs does not allow for sufficient elimination of exceptional frogs—those much more or less resistant than the average. It has never found adherents in the United States.

In addition to applying a quantitative method to digitalis assay, Focke experimented with leaves from different sources, of different ages, wild and cultivated, the effect of moisture on deterioration, and the seasonal and temperature effect on the frog. In all, while not much of his work was purely original, he added considerable to our knowledge of this drug and its standardization.

Santessen[17] used another modification of frog-heart method in that he observed the effect on the heart, but did not expose it until the heart had practically reached systolic standstill. He recognized the difficulties of physiological standardization and the importance of the factor of individual resistance as well as the general factors which affect results in such a method.

Hatcher[18] first adopted the systolic stoppage of the heart in one hour as the method and end-point for assay purposes, but later wrote on his experiments with the cat method, adopting the technic of Bennefield, that of slowly injecting the material into the vein over a period of 90 minutes, death to take place at that time. This is the first suggestion of the practical use of this method for quantitative assay and it may be said that, while there seems little to recommend it, the method has appealed to some as of considerable importance. One of the arguments for it,

or rather used against the M. L. D. frog-heart method, is that a product may possess toxic principles which are of no therapeutic value but which appear valuable by strictly M. L. D. methods. It should be noted, however, that the cat method is nothing more than a toxicity test with the disadvantage that the characteristic systolic stoppage cannot be observed to identify the cause of death.

Hatcher, himself, admitted that he found unaccountable variations of about 50 percent and his tables show even greater variation than he admitted.

Robinson and Wilson,[19] in some experiments to establish the character of the digitalis action on the cat's heart, observed a total variation of 100 percent in the lethal dose.

Reed and Vanderkleed,[20] objecting to the use of frogs because of the number of variable factors concerned, proposed the M. L. D. of guinea pigs as a quantitative method. They have, however, failed to prove that guinea pigs have a constant resistance and that death results from a direct action on the heart. It is another M. L. D. method with no technic to confirm the cause of death, such as is available in the frog-heart methods.

The work of Sollmann and others[21] in showing the influence of temperature on the toxicity of the digitalis series to frogs is well worth noting and is highly important. This variable factor, however, is offset in both the frog-heart methods by the use of the standard for comparison in every assay, thus eliminating this as well as other uncontrollable factors, such as climate and season and the species and weight of the frogs.

Heintz,[22] in 1912, recognizing the limitations of the various physiologic tests proposed, suggested applying not one but several tests, including the M. L. D. and M. S. D. on frogs, the M. L. D. on mice, and the pressor action on the circulatory system of rabbits and cats. The toxic dose on mice is by internal administration with food in pill form. His proposition has received little comment.

Krogh[23] used the isolated frog's heart and determined the lowest concentration of the drugs which would arrest the spontaneous rhythm. He considered the method accurate within 10 percent.

One other proposed method remains to be noted, namely, that

of Pittenger,[24] who suggested the use of gold fish, which are particularly susceptible to the influence of poisons in water. It is probably the simplest method heretofore proposed, but it appears to have gained insufficient recognition for criticism. It is another M. L. D. method and allows of no means of identifying the poison that causes death.

Of the methods quoted, only the M. L. D. and M. S. D. on frogs, and M. L. D. on guinea pigs and cats, are practiced in the United States. The M. S. D. on frogs is the one suggested in the U. S. P. IX as adapted to the standardization of this important series of drugs.

The cat method, as stated before, is purely a toxicity test and can be classed with that on guinea pigs as objectionable because death is almost invariably due to paralysis of the respiratory centers, and, therefore, not directly a measure of the heart tonic value.

As stated by Edmunds and Hale, there is little to choose between the M. L. D. and M. S. D. on frogs. To one who has been accustomed to the former, however, it has three advantages over the M. S. D. method: first, in the use of a larger number of frogs with less work and actual time involved; second, in the elimination of the factor of slow absorption; and third, in the fact that the end-point is not obscured by rough handling such as by the pithing and laying bare of the frog's heart. At the same time it has the only advantage claimed for the M. S. D. method in that the frog's heart can always be examined to verify the identity of the toxic principle.

THE PITUITARY GLAND

While the extracts of this gland have widely different effects, such, for example, as the pressor, diuretic, galactagogue, cathartic, and oxytocic actions, no undisputed chemical evidence has been brought forward to demonstrate the presence of more than one active constituent—a substance which acts on plain muscular tissue and is responsible for all the phenomena noted.

As assay methods, only three have been proposed and of these only two are generally used, one being official in the 9th Revision of the U. S. P.

The first method proposed is that by Dale and Laidlaw,[1] who

in 1912 described the method which with some modifications is now official. It consists, in brief, in using the isolated uterine muscle from a young guinea pig of not to exceed 350 Gm. weight. One horn is removed, suspended between a fixed and a movable attachment in artificial blood plasma (Locke's solution) heated to body temperature, and the solution to be tested is thoroughly mixed with the Locke solution to make a homogeneous mixture in contact with the uterine muscle. It is claimed that when all the conditions are rigidly followed the contractions of the uterus will vary according to the amount of active principle present in the solution.

There are many factors affecting the sensitiveness of the muscular tissue used in the test and, therefore, affecting the quantitative accuracy of the method, such, for example, as the size, age and condition of the pig, the temperature changes of the solution in contact with the specimen, and the presence of foreign substances in the Locke's solution or in the pituitary extract.

The method has been later described by a number of authors, in each case with some slight variation in technic.

Fühner[2] applied this method in attempting to prove the separation from the gland of four active constituents with different properties. One of these constituents was considered to be the active principle, but others had similar properties less strongly pronounced.

Heidelberg, Pittenger and Vanderkleed[3] consider the oxytocic to be the only method practicable for quantitative assaying, describing in minute detail the apparatus and technic used by them.

Guggenheim[4] used the rat uterus with approximately the same technic. There was no exceptional contraction observed, but after applying hypophysis extract to the uterus it maintained a steady contraction instead of the normal rhythmic contractions.

Roth[5] described the Dale method in minute detail with his modifications and concluded, on the basis of considerable experimentation, that it is the most satisfactory assay method, he having also applied tests on the intact uterus and the blood pressure. The intact uterus is so rarely used that it is not regarded as a distinct method.

For a standard in carrying out the uterine contraction assay Roth proposed and used a solution of histamine which acts similarly to pituitary on the uterine muscle but which more often lowers than raises blood pressure. The final dilution of histamine found best suited in most cases is 1 in 20,000,000, which, in comparison with two of the best known commercial preparations, diluted 1-15000 caused equal contractions. No tracings were submitted to substantiate these statements.

For the pressor test, histamine not being applicable, a commercial preparation was selected for comparison with the others.

The blood pressure method was proposed by Hamilton[6] the same year that Dale's method was published.

Disregarding, because of expediency rather than for any other reason, the physiological effects of pituitary extracts other than that on the circulatory system, this author proposed a pressor test by which the activity of the extract could be measured with considerable accuracy.

The dog anesthetized with chloretone was used in the same general way as for standardizing suprarenal gland extracts, comparison being made between the sample and a standard prepared from the dried, defatted, powdered posterior lobe. The standard material was prepared in considerable quantity in order to represent an average product. The amount to be injected at one time was specified as 1 cc of the solution to be obtained by dissolving the soluble part in acidulated water, using 1 Gm. to 1000 cc.

The method was described in greater detail by Hamilton and Rowe,[7] who critically analyzed the two methods, pointing out the discrepancies in Roth's results and particularly calling attention to the failure of the oxytocic method as a measure of pressor action of pituitary extracts because of the use of histamine as a standard, since the latter is usually not a pressor agent. It is also questionable as a standard because its clinical action is not identical with that of a pituitary extract. The unqualified use of histamine as the standard was also criticised by Pittenger and Vanderkleed,[8] and by Pittenger,[9] who have not found different lots of histamine equally active, nor have the solutions been proved to be stable.

The U. S. P. 9th Revision Committee gave no details of the

test, but directed attention to the method described by the Hygienic Laboratory. The strength of the official solution, however, has been specified as equal to that of histamine 1 to 1000, a value estimated by different authors as being from 10 percent to 40 percent as strong as a good commercial preparation. This has been noted by Pittenger,[9] Hamilton,[10] and Eckler,[11] and the result of the statement is that no official preparation has appeared on the market, since strict adherence to this standard would in some cases lower the activity of the preparations considerably below the present high standard.

The objections to the oxytocic method other than those concerning the character of the standard and the activity of the official product refer principally to the fact that the uterine muscle is so sensitive to other stimuli than that specific for pituitary extracts that uniform quantitatively accurate results are not obtainable, the reaction being rarely proportional to the amount of extract applied.

The principal objection advanced against the pressor test is that the dog, while least subject of all animals to this fault, does not remain sensitive to repeated injections, but becomes progressively less responsive after the first two or three. This, however, has not proved a valid objection in the work of the author.[7,9]

The third method proposed is that of Spaeth,[12] who uses the melanophores of *F. heteroclitus*. "As a result of this work it appears that the melanophores of Fundulus and probably of all other teleost fishes must be considered functionally modified smooth muscle cells." These melanophores or pigment cells of the fish are a part of the scales.

In the assay method these scales are placed in the pituitary solution and the resulting contraction is evidenced by the apparent bleaching out of the pigmented portion of the scales. This effect is also produced by potassium chloride in 0.1 *N* solution and it is proposed that a dilution of this solution shall be used as a standard for comparison.

The standard test solution is a mixture of 1 part 0.1 *N* KCl and 2.5 parts 0.1 *N* NaCl. The solution of pituitary extract is to be mixed with an equal amount of 0.2 *N* NaCl—a dilution which Spaeth suggests as "a uniform standard for pituitary extract." As the author has had no experience with the method—

the *F. heteroclitus* not being available in inland towns—it will not be discussed except from its superficial aspects.

It is not an illogical test in that the same general effect of pituitary extracts as that tested in the other methods is made use of, namely, the constricting action on smooth muscle—an effect which should be fairly transferable from any one kind of tissue to another. The use of KCl as a standard instead of a standard pituitary product is no more illogical than the use of histamine in the oxytocic test.

It is improbable that the use of a test object not easily obtainable in every locality would ever appeal to the average investigator unless the method were pre-eminently satisfactory. This has not been demonstrated either by the originator of the method or by any other investigator.†

The principal points to be considered in comparing the practicability of the first two methods (omitting the third, since no data on it are available), are as follows: The pressor test measures the constricting effect of pituitary extract on smooth muscle and has been found free from many of the objectionable features of the oxytocic effect, such as its supersensitiveness and lack of uniformity in results. The dog, contrary to Roth's statement (which he failed to prove), does not become progressively less reactive to pituitrin if the proper technic is followed.

THE SUPRARENAL GLAND: ADRENALIN

The physiological standardization of extracts of this gland depends entirely on its constrictor effect, and the assay method now used to the exclusion of all others is the constricting action on the arterioles causing an increased blood pressure. In fact, so completely has this effect taken the place of other reactions that the use of any other test is not considered.

Oliver and Schafer,[1] in 1894 and 1895, noted the marked blood-pressure-raising action of these extracts, as did also a number of others about the same time.

Von Fürth, in 1898,[2] carrying out pharmacologic tests in conjunction with his chemical experiments, observed that when a

†Unofficial reports from the Hygienic Laboratory are to the effect that this method was found to be inaccurate and impracticable.

substance isolated from the gland was intravenously administered in a dose of 0.000025 Gm. to a rabbit the blood pressure rose 114 to 116 mm.

Among others who wrote of this reaction is Gottlieb, whose contribution[3] is noteworthy because of the tracings showing the action of suprarenal extract on the circulatory system of a dog, the blood pressure of which had been reduced to zero by paralyzing the heart with potassium nitrate.

This combined action on the heart and circulatory system is the effect which forms the basis of the method proposed by Houghton[4] in a paper read before this Association in 1901.

The method in brief consists in the use of an anesthetized dog, the injections being made intravenously and the results recorded from the carotid artery by means of a kymograph. The injections can most conveniently be made into one of the femoral veins and the record made and preserved on a sheet of blackened paper on the revolving drum of the kymograph. Tracings obtained in this way are shown in which the rise in blood pressure varies directly with the amount of active agent injected.

My personal experience with this method began in 1900, at which time the standard used for comparison was a carefully prepared and preserved extract of the gland so diluted before injection that the rise in blood pressure would not in general be greater than 20 to 30 mm.

In 1902, in a second paper by Houghton,[4] the method was amplified and the active constituent, adrenalin, was proposed as the standard, with the test dose for the average dog 1 cc of solution containing 0.00001 Gm. of adrenalin chloride. This is by no means a minimum active dose, but it was selected as the dose from which the reaction was most sensitive to minute changes. Thus it is easy to distinguish a difference of 5 percent more or less than this amount when injected into an average dog, and it therefore permits of standardization within these limits of error.

No material change from this technic was proposed by anyone until the Revision Committee of the 9th U. S. P. included the suprarenal gland among the drugs for which a physiological test was proposed. To the casual reader the two methods are identical, but to the operator there are several points of difference which

have received critical comment by Hamilton,[5] who pointed out sources of error in the described technic.

Cameron,[6] in 1906, after trying various other methods, chose the blood pressure method as the most convenient and reliable for the purpose of standardizing suprarenal products.

Hunt,[7] and Sollmann and Brown,[8] using the same general method, assayed and reported on several commercial samples.

Crawford[9] reviewed the literature and gave minute details used in applying this method, but suggested no additional methods.

Läwen,[10] using the same kind of a reaction, but applied by perfusion through the blood-vessels of the frog, demonstrated the constricting effect, but found the animals to vary considerably in their response.

He observed the effect of a mixture of cocaine and suprarenin, as Braun had first noted, to be a localization of the former and thus prevention of its general action.

Ehrman[11] applied the reaction to the pupil of the enucleated frog's eye, the constricting effect in this case being on the iris, thus causing a measurable dilatation of the pupil. He considered the method equally accurate with other applied methods, but his results show that a dilution of adrenalin corresponding to the commonly used test solution, namely, Solution Adrenalin Chloride 1-1000 diluted 1 in 100, *i. e.* 0.00001 Gm. per cc, was inactive.

In this historical account of physiological standardization no attempt has been made to have the references exhaustive, because many of the workers along these lines have made no attempt to apply the test quantitatively. On the other hand, a number of the authors quoted have not contributed directly to the development of drug standardization, but their work has been very helpful in pointing the way and for that reason is too important to be omitted.

In collecting the bibliography I have been greatly assisted by consulting the records of a number of authors who have in some cases had access to more extensive libraries than were available to me.

It is evident from a study of the collected references abstracted that there has been no orderly development of the methods offi-

cial or unofficial now in use to assay these important drugs. In two cases—Cannabis Sativa and Adrenalin solutions—there has been only one method for each adopted for general use, and in both cases that method made use of one of the first typical effects observed. In the other three cases—ergot, the digitalis series, and pituitary gland extracts—two or more methods are in common use and there seems no way to reconcile conflicting opinions.

The reason for this is that in no case is any opinion wholly right or wholly wrong and no one is inclined to give up a method which in his hands has given fairly satisfactory results for one which does not appeal to him as being either more logical or more accurate.

If it were possible to check up by clinical tests the results obtained by the different methods of pharmacologic assay—if, for example, it were possible to determine which is the better of two samples of pituitary extract, alike by one method of assay and different by another—then differences of opinion as to the adaptability of any particular method would vanish. This, while apparently a simple means of eliminating discord, has never been worked out in practice, and we seem no nearer to a satisfactory solution of the problem than at any time in the past.

How serious these differences of opinion are as to which method of assay is correct the following quotation from a letter which passed between two state universities will serve to illustrate: "Permit me to call your attention to the fact that the studies of Roth and Edmunds and many other workers merely relate to the toxicity (of digitalis). Furthermore, in this connection the U. S. P. suggests a standard based on toxicity which, in the opinion of Hatcher and many other workers, is not the measure of the therapeutic value of the drug. A sample may be toxic and high in vaso-constrictor properties, and at the same time be absolutely contra-indicated in 90 percent of the cases where digitalis is called for."

The above quotation is evidence that the official recognition of a method is not sufficient to obtain its general recognition, and shows how valuable some clinical evidence would become if it were practicable to obtain it.

The history of Physiological Standardization to date is a record of wonderful development in the face of many discourage-

ments. The opposition has, however, largely broken down and thus the way is cleared for still greater achievement. With the active coöperation of the clinician much might be accomplished which under present conditions is next to impossible.

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[2]Von Fürth, *Zeitschrift g. phys. Chemie*, 26, 15 (1898).
[3]Gottlieb, *Arch. Exp. Path. Pharm.*, 43, 286 (1899).
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**Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 195, 1919.**

(Reprinted from the *Journal of the American Chemical Society*, Vol. XLI, No. 1, pp. 125-129, January, 1919.)

DIGITALIS LEAVES: EFFECT ON ACTIVITY OF TEMPERATURE IN DRYING.

By Herbert C. Hamilton.

(From the Medical Research Laboratories, Parke, Davis & Co., Detroit, Mich.)

Since the first attempt to standardize digitalis leaves and the extracts, it has been observed that they vary greatly in activity.

Bennefield,[2] in 1881, using a method almost identical with that suggested in the 9th Rev. U. S. P. for standardizing the digitalis series of heart tonics, found a variation of about 500% in the activity of tinctures from digitalis leaves from various parts of Germany.

Bührer,[3] in 1900, found a difference of 400% in the activity of some fluidextracts. Fränkel[4] found variations of 300 to 400% in tinctures and infusions. Edmunds,[5] in 1907, tested 17 commercial tinctures and found a variation of 400%.

Many other similar results have been recorded, in some cases the reason being assigned to climate, soil, variety, or the locality from which the leaves were obtained.

Focke[6] observed that wild digitalis is more toxic than the cultivated and the second year's growth than the first. He also observed that the leaves gathered at seeding time are less active than when collected earlier. He was the first to record his observations as to the causes of deterioration and the effect of light and heat in drying the leaves. The former is negligible, but he considered that when dried in the air in the ordinary way without special care in preserving, the activity is soon largely lost. This he considered to be due to the moisture content, which per-

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[2]Bennefield, "Ueber Digitalis Tincturen," Inaug. Diss. Göttingen, 1881.
[3]Bührer, Inaug. Diss., Basel, 1900.
[4]Fränkel, *Therap. Gegenw.*, 43, 106 (1902).
[5]Edmunds, *J. Am. Med. Assoc.*, 48, 1744 (1907).
[6]Focke, *Arch. Pharm.*, 245, 646 (1907).

mitted the enzymes and ferments of the leaves to remain in an active state, and to their action in breaking down the sensitive glucosides to less active substances. His remedy is to heat the leaves rapidly to a temperature not to exceed 100°, drying to a moisture content of about ½ of 1% and preserving in dark air-tight jars.

Tordes,[1] in 1867, claimed that digitalis from the vicinity of Strassburg was better than that from other localities because of the careful selection, drying and preserving of the leaves. They used leaves of the second year's growth only, first dried in the shade, then in an oven at a temperature not over 40° C. The leaves were then kept in tin or glass containers away from light and moisture.

Sharp and Lancaster,[2] in a series of careful experiments, showed that digitalis leaves, not specially dried but kept dry, retained their activity for 11 years, while the fluid preparations began to deteriorate between the thirteenth and fifteenth months. They also observed that leaves of first-year plants were intensely bitter and probably very active.

Hatcher and Eggleston[3] found that old samples of digitalis leaves and tinctures, neither of them specially preserved, were not much below their original activity. They concluded that fluid preparations containing not less than 50% alcohol do not deteriorate to any considerable degree. This, however, is not in accord with the conclusions of most investigators, although the higher strengths of alcohol are in general much better than the lower for preserving the activity of digitalis preparations.

Houghton and Hamilton,[4] in a series of tests and retests of digitalis extracts, showed that none of them were free from the fault of deterioration, but the higher percentages of alcohol appeared to not only more completely extract but also more thoroughly preserve the active principles.

On the supposition that the strong alcohol destroys the active ferments, these results are in accord with the results of Perrot and Goris,[5] who published a method by which the enzymes could be destroyed, with the subsequent complete preservation of the

[1]Tordes, *Gaz. Med. Strassburg*, 27, 191 (1867).
[2]Sharp and Lancaster, *Pharm. J.*, 32, 102 (1911).
[3]Hatcher and Eggleston, *Am. J. Pharm.*, 85, 203 (1913).
[4]Houghton and Hamilton, *Ibid.*, 81, 461 (1909).
[5]Perrot and Goris, Abs. in *La Presse Medicale*, 17, 776 (1909).

drug in its original activity. This was accomplished by subjecting the drug to the vapors of boiling alcohol, after which it was dried in the air. Such precautions, however, seem unnecessary in view of the results with old samples of digitalis obtained by the different investigators quoted, especially Hatcher, who found high values in 25-year-old leaves, and Sharp and Lancaster, who found 11-year-old drug to have lost little of its activity.

The writer recently had occasion to extract and test a sample which had been in the possession of Northwestern University for 25 years. Its activity was fully 150% of that of the average drug at present obtainable.

Recently the subject of drying digitalis leaves has come up in connection with the samples of this drug grown wild in Oregon and submitted to the Government for the Medical Department of the Army. It was stated that unless the drug was dried in an oven, at 75° to 90° C., it was practically worthless.

This statement being so entirely at variance with the opinions commonly held, some experiments were inaugurated to demonstrate its correctness. Unfortunately, there was not available a sufficient amount of the growing digitalis leaves to make the experiments conclusive, but the results are apparently of sufficient importance to be published.

From some previous experiments, unpublished, it had been observed that the fresh leaves extracted with 95% alcohol had a higher degree of activity than the average digitalis on the market, and apparently the tincture, so prepared, was more active than that prepared from a part of the same lot of leaves dried before extraction.

The following experiments were, therefore, planned and carried out. Fresh leaves were gathered from the flowering and fruiting plants in July, divided into three equal amounts and extracted as follows:

First: Extracted immediately with 95% alcohol for the moistening and then with 70% alcohol to complete exhaustion.

Second: Dried in an oven at temperatures ranging between 75° and 90° C., then extracted with 70% alcohol. The drying covered a period of about 5 hours.

Third: Dried in the air and partly in the sun over a period of 4 days, then extracted with 70% alcohol.

The tinctures were made to the same amount on the basis of the weight of the oven-dried lot, which was considerably less than that of the air-dried sample.

Only two lots of drug were available, one being the official variety *digitalis purpurea*, and the other a non-official variety.

The results of assays are as follows, the method of testing being the M. L. D. method originally applied by Houghton.[1] The correctness of the end result was in every case checked by examining the heart of the dead frog to determine whether death occurred with heart in systole—the characteristic position from digitalis poisoning.

These results coincide with those previously obtained in that the fresh drug has greater toxicity than the dried. The experiments also show that the high temperature employed in the oven caused a greater immediate deterioration than the slower drying at the season temperatures.

Table I. Digitalis Purpurea from a Flower Garden in Detroit.

Not dried.			Oven dried.			Sun / Air dried.		
Weight.	Dose.	Result.	Weight.	Dose.	Result.	Weight.	Dose.	Result.
22	0.004	Alive	16.5	0.010	Alive	19	0.010	Dead
22	5	Alive	10.5	0.012	Dead	19	0.012	Dead
22.5	6	Alive	16.5	0.015	Dead	19.5	0.015	Dead
22.5	7	Alive	14	0.020	Dead	20.5	0.018	Dead
23	8	Dead	13.5	0.025	Dead	20.5	0.022	Dead
23.5	0.0045	Alive	23	0.008	Dead	24	0.007	Alive
25	55	Alive	24	0.010	Dead	24.5	0.008	Alive
25	65	Alive	24	0.012	Dead	24.5	0.009	Dead
25	.75	Dead	24	0.014	Dead	25	0.010	Dead
26	0.0090	Dead	25	0.016	Dead	26	0.011	Dead
27	0.0055	Alive	20	0.006	Alive	22	0.007	Alive
27	65	Alive	20	0.007	Alive	22.5	0.008	Alive
27.5	75	Alive	21	0.008	Alive	23	0.009	Dead
29.5	85	Dead	21	0.009	Alive	24	0.010	Dead
30	0.01	Dead	21	0.010	Dead	26	0.011	Alive
27	0.0070	Alive	24.5	0.008	Alive	25.5	0.008	Dead
29.5	75	Alive	25	0.009	Dead	25.5	0.009	Dead
29.5	75	Alive	25.5	0.010	Alive	26	0.010	Dead
30.5	80	Dead	25.5	0.011	Dead	26	0.011	Dead
33	80	Dead	25.5	0.012	Dead	26	0.012	Dead
0.007 killed none of 2			0.008 killed 1 of 3			0.008 killed 1 of 3		
0.0075 killed 1 of 4			0.009 killed 1 of 2			0.009 killed 3 of 3		
0.008 killed 3 of 3			0.010 killed 2 of 4			0.010 killed 4 of 4		
			0.011 killed 1 of 1			0.011 killed 2 of 3		
			0.012 killed 3 of 3			0.012 killed 2 of 2		
Activity		153		100			133	
H. T. U..........		9+		6			8	
M. L. D..........		0.008		0.011			0.009	
Activity in terms of undried drug ...		100%		72			81	

[1] Houghton, *J. Am. Med. Assoc.*, 31, 959 (1898).

Further experiments are planned to demonstrate whether the oven-dried drug is more stable than the air-dried sample, but this point could not be considered, as not sufficient leaves were available to make the experiment conclusive. Such experiments should be continued over a period of not less than three years.

In further consideration of the previously mentioned criticism against the Oregon air-dried leaves it should be noted that the method of testing was also called in question. On this point, while there is a wide divergence of opinion as to which of several methods shows the real value of the drug, there is quite general agreement on the frog as the test animal, and until clinical evidence is brought forward to negative the results it is logical to assume that the death of the frog with heart in systole, or the stoppage of the heart in systole in one hour, is a satisfactory measure of the activity of a digitalis preparation. Compared with the guinea-pig method of Reed and Vanderkleed[1] and the Hatcher cat method,[2] both of which are purely toxicity methods, the frog methods have the advantage that deaths from other than digitalis poisoning are eliminated and form no part of the record.

TABLE II.—DIGITALIS LEAVES NON-OFFICIAL FROM A GARDEN ON GROSSE ILE, MICH.

Not dried.			Oven dried.			Sun / Air dried.		
Weight.	Dose.	Result.	Weight.	Dose.	Result.	Weight.	Dose.	Result.
17	0.004	Dead	20	0.004	Dead	23.5	0.002	Alive
18.5	0.005	Dead	27	0.005	Dead	25	0.003	Dead
19.5	0.006	Dead	23.5	0.006	Dead	25	0.004	Dead
27.5	0.007	Dead	23.5	0.007	Dead	25.5	0.005	Dead
18	0.0010	Alive	21	0.0010	Alive	19.5	0.0010	Alive
19	0.0020	Dead	22	0.0020	Alive	20	0.0015	Alive
19	0.0030	Dead	22.5	0.0030	Alive	20.5	0.0020	Alive
19	0.0040	Dead	25	0.0040	Alive	21.5	0.0025	Alive
20	0.0050	Dead	27.5	0.0050	Dead	22.5	0.0030	Alive
18	0.0010	Alive	23	0.0035	Dead	30	0.0025	Alive
19.5	0.0015	Dead	23	0.0040	Dead	28	0.0030	Dead
20.5	0.0020	Dead	23	0.0045	Dead	28	0.0035	Dead
21	0.0025	Dead	23.5	0.0050	Dead	29	0.0040	Dead
21	0.0030	Dead	23.5	0.0060	Dead	30	0.0045	Dead
			22.5	0.0020	Alive			
			23	0.0025	Alive			
			25	0.0030	Alive			
			27	0.0033	Dead			
			27.5	0.0040	Dead			

	Not dried	Oven dried	Sun / Air dried
M. L. D.	0.0015	0.0035	0.0030
H. T. U.	44	19	22
% activity	730	310	370
Activity compared with undried...	100%	42	50

[1]Reed and Vanderkleed, *Am. J. Pharm.*, 80, 110 (1908).
[2]Hatcher and Brody, *Ibid.*, 82, 360 (1910).

One may conclude, therefore, that oven drying has no advantage over a reasonably rapid air drying of digitalis leaves, and that the drying causes a marked deterioration, no products more highly toxic than those present in the crude drug having been developed during the process of drying.

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 196, 1919.

(Reprinted from the *Journal of Industrial and Engineering Chemistry,* Vol. 11, No. 2, page 139. February, 1919.)

AN EFFICIENT LABORATORY FUNNEL FOR FILTERING NEUTRAL LIQUIDS, ESPECIALLY THE VOLATILE ORGANIC SOLVENTS.

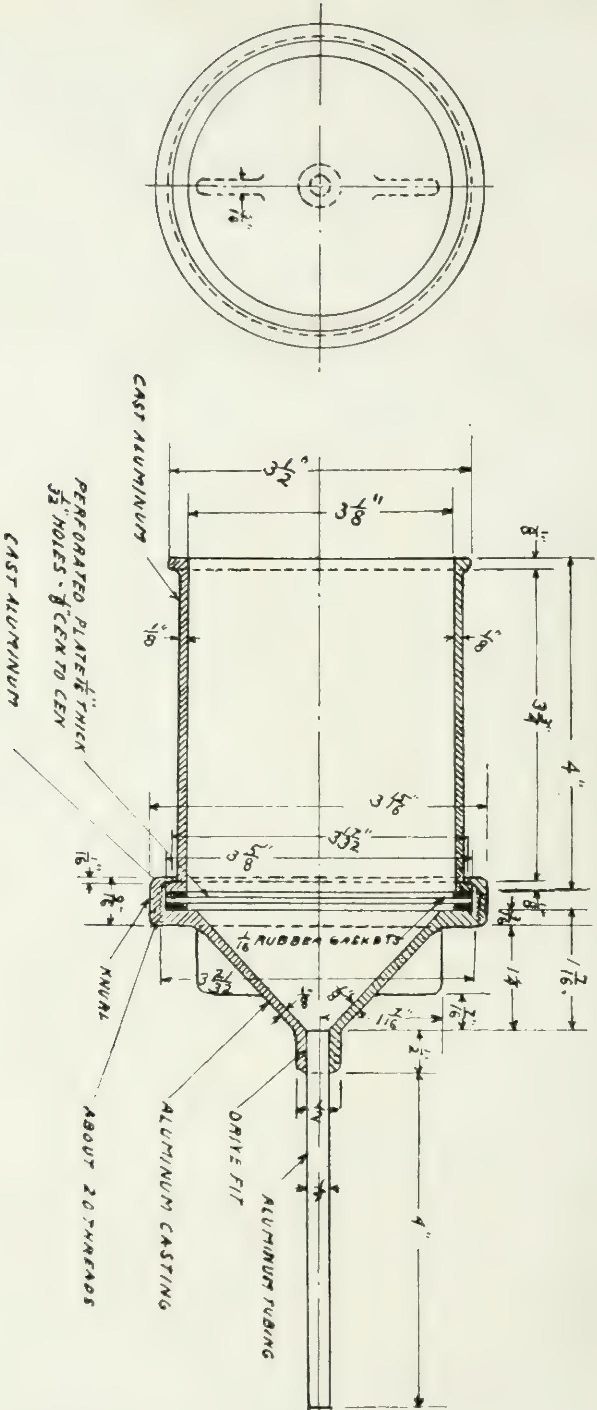
By T. B. Aldrich

(From the Medical Research Laboratories, Parke, Davis & Co., Detroit, Mich.)

Those who have had occasion in the laboratory to collect precipitates suspended in ether, chloroform, acetone, alcohol, or mixtures of these organic solvents, especially by forced filtration, have no doubt at times experienced trouble when using the ordinary funnel and especially the Buchner funnel.

In the case of aqueous solutions, the filter paper, after being moistened and pressed closely to the funnel for the purpose of removing bubbles of air that interfere with filtration, adheres closely to the glass and filtration is ordinarily rapid, while in the case of ether, for example, the solvent being volatile passes away rapidly, and channels and spaces are formed between the paper and the funnel, the paper tending to lift from the glass. This is especially true when filtration is interrupted for a short time; in any case the formation of channels and spaces interferes with rapid filtration. When the Buchner funnel is employed these troubles are aggravated, since the precipitate and liquid tend to creep under the paper filter, especially if continuous vacuum is not maintained, thus requiring a second filtration.

The advantage of the funnel to be shortly described lies in the fact that the filter paper is clamped securely between two plates by a screw thread so that it cannot lift even when the filtration is interrupted and thus allow the formation of channels and passages for the liquid and precipitate underneath. There is no necessity for second filtrations, and a more rapid filtration is also effected by this arrangement.



CAST ALUMINUM
PERFORATED PLATE 1/16" THICK
1/32" HOLES - 1/8" CEN TO CEN
CAST ALUMINUM
1/16 RUBBER GASKETS
KNURL
ABOUT 20 THREADS
ALUMINUM CASTING
DRIVE FIT
ALUMINUM TUBING

FIG. 1

DESCRIPTION

The funnel is of aluminum and consists essentially of four parts having the following dimensions: a cast hollow cylinder approximately 4 in. high, 3½ in. in diameter, walls ⅛ in. thick, with a small flange at the top and a heavier one at the bottom, both being on the outside; a plate 1/16 in. thick, perforated with 1/32 in. holes (⅛ in. center to center) extending to within ⅛ in. of the circumference; a ring 9/16 in. wide and ⅛ in. thick, threaded on the inside and milled on the outside, having a shoul-

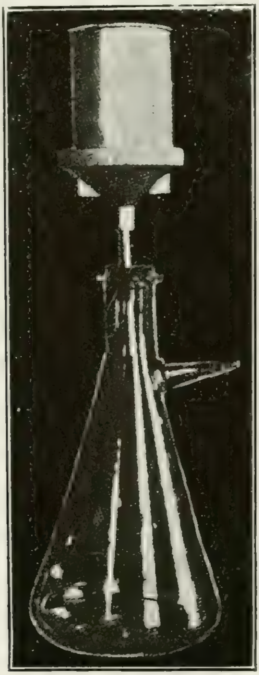


FIG. 2

der (or offset) on the upper inside which engages the lower upper surface of the lower shoulder of the cylinder, this latter preventing the ring from passing over the lower end of the cylinder; a cone-shaped lower portion with stem having also a flange at the top ¼ in. thick and threaded on the outside so as to mesh with the threads on the inside of the ring.

The lower flange of the cylinder, the plate, and the top of the flange of the cone have approximately the same outside diameter, as must also the gasket and filter paper to be used. The upper flange on the cylinder is not essential, except for strengthening the same; but the lower is essential as a shoulder of resistance for the ring when the cylinder, plate, cone, gasket, and paper are pressed together, by meshing the ring with the cone.

In preparing the funnel for filtering it is only necessary to have the lower flange of the cylinder, the filter paper (placed on top of the plate), the perforated plate, the gasket (placed between the flanges of the cone and plate), and the flange of the cone flush with one another, pass the ring with flange or shoulder uppermost over the top of the cone, screwing as tight as necessary to secure a perfect joint. A key made of hard wood and carrying two slots which engage two offsets shown in the drawing and plate, opposite each other on the lower part of the cone, facilitates the operation of tightening and loosening the parts.

Another smaller funnel having approximately the dimensions $2\frac{3}{4}$ in. x $1\frac{1}{2}$ in. but in which the perforated plate is not separate but a part of the cone, has the advantage that the rubber gasket is eliminated. In this funnel the perforated plate is depressed 1/16 in. below the upper edge of the cone flange and brazed in. Here it is only necessary to place the filter paper on the top of the perforated plate and screw the ring over the cone.

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 197, 1919.

(Reprinted from the *Journal of the American Chemical Society*, Vol. XLI. No. 2. February, 1919.)

STUDIES ON PEPSIN. I. CHEMICAL CHANGES IN THE PURIFICATION OF PEPSIN.[1]

By Lewis Davis and Harvey M. Merker.

(From the Medical Research Laboratories, and the Department of Glandular Extracts, Parke, Davis & Co.)

The question of the chemical composition of pepsin has occupied the attention of a number of investigators. Following the classical researches of Pawlow[2] and his pupils, Pekelharing[3] appears to have been the first to undertake purification of the enzyme. This investigator prepared a light yellow powder which, while readily soluble in dilute acids and sodium chloride solution, dissolved with difficulty in water but showed strong peptic activity. It gave reactions for albumin, but was believed to contain a soluble phosphorus compound as an impurity. On boiling pepsin solutions, Pekelharing obtained a nucleoproteid and was able, under certain conditions, to separate an albumose.

Nencki and Sieber,[4] using as initial material juice obtained through gastric fistula in dogs, claim to have secured an active pepsin preparation through precipitation which is free from albumin. At the same time, they consider the precipitate of transparent granules containing chlorine which they obtained by strongly cooling the gastric juice to be a chemical individual, and, in all probability, the true enzyme. They also submit analyses to support their contentions. Pekelharing,[5] in a later investigation, in which he employed the artificial gastric juices extracted from several hundred hog stomachs by his previous method, and also the juice obtained from gastric fistula in dogs, disproved this view. He found pepsin to be free from phosphorus and

[1]Read before the Biological Section of the American Chemical Society at the Cleveland meeting, September 12, 1918.
[2]Pawlow, *Centr. Physiol.*, 1888; *Ergebnisse Physiol.*, 1902, i, Part I, 246.
[3]Pekelharing, *Z. physiol. Chem.*, 22, 233 (1897).
[4]Nencki and Sieber, *Ibid.*, 23, 291 (1901).
[5]Pekelharing, *Ibid.*, 35, 8 (1902).

to contain no nucleoproteid, but the analyses of his preparations showed no constancy in results.

That a protein-free pepsin solution having digestive action is possible, has also been maintained by Schrumpf.[6] The latter prepared a Büchner-pressed extract of hog stomachs, clarified by filtration, and dialyzed against running water. The dialysate thus obtained was precipitated by addition of cholesterin in alcohol-ether solution, filtered, the precipitate redissolved in water, and the suspension finally clarified by a Kitasato candle. The clear filtrate, while giving none of the protein reactions, still showed powerful digestive activity.

The amino-acid constituents of pepsin have been investigated by Hugounenq and Morel[1] using an autodigested hydrochloric extract of hog stomachs. They conclude that an extract of pepsin contains a number of monoamino-acids in the free state, probably formed in the autodigestion. Glycocoll, aspartic and glutaminic acids, and also histidin, they found to be absent in the material examined.

It is thus readily apparent that, as with other enzymes, the chemical nature of pepsin is still an open question. Nearly all of the above investigators have based their conclusions on crude preparations, undoubtedly containing admixed or combined impurities. Seemingly no attempt has been made to prove, by quantitative measurements of the proteolytic activity, that an actual purification has taken place where such is mentioned. The present investigation was undertaken by us to determine what changes take place in the purification of pepsin, with the view of possibly throwing some light on the chemical nature of the enzyme.

EXPERIMENTAL PROCEDURE.

METHODS.—As basic material for purification, a composite lot (consisting of a number of different samples) of 1:2000 commercial pepsin was employed. Sufficient stock of this mixture was reserved to permit the preparation of all of the various strengths of the enzyme given below. The weaker samples (up to 1:18000) were prepared by fractional precipitation of a 20% aqueous solution, while the more active strengths were obtained

[6]Schrumpf, *Beitr. Hofm.*, 6, 396 (1905).
[1]Hugounenq and Morel, *Compt. rend.*, 147, 212 (1908).

Table I.—Analyses.

Proteolytic strength (U.S.P. IX)	Total mineral matter. %	Phosphoric acid as P_2O_5 %	Calcium as CaO. %	Chlorides as NaCl. %	Total sulfur. %	Percentage of nitrogen in: Total	Coagulable protein.	Proteoses.	Peptones.	Amino acids.	Optical rotation a_D at 24°	Reaction C_{H+} at 23°
1:2000	5.37	1.58	0.26	1.19	0.63	12.93	1.15	0.73	7.37	4.39	—2° 58′	....
1:5500	4.31	1.42	0.32	Trace	0.70	12.60	1.41	1.76	4.78	4.04	—2° 0′	....
1:6000	3.34	1.03	0.46	Trace	0.81	13.41	1.43	2.10	4.91	3.15	—2° 6′	....
1:10,000	3.31	1.42	0.35	Trace	0.89	13.55	1.63	3.00	3.73	3.09	—2° 24′	....
1:12,000	2.31	1.28	0.29	Trace	0.63	12.95	2.33	3.09	3.41	2.75	—2° 10′	....
1:18,000	2.84	1.47	0.71	Trace	1.50	13.47	3.16	3.62	2.68	2.10	—2° 30′	....
1:21,000	2.38	1.29	0.58	Trace	0.82	12.57	3.69	3.91	3.73	1.45	—2° 0′	....
1:24,000	2.84	1.27	0.52	Trace	0.77	12.64	3.98	4.10	0.96	1.35	—2° 4′	2.5×10^{-4}
1:28,000	1.86	1.09	0.53	None	1.62	13.72	4.39	4.32	0.78	1.22	—2° 20′	4.0×10^{-5}
1:40,000	2.01	0.47	1.01	None	1.50	13.77	8.34	4.43	....	0.61	—2° 30′	6.0×10^{-7}

by salting out the former, filtering and dialyzing. In each case, the final purified material was dried to a constant moisture content of about 5% and sealed. Assays for proteolytic power were then carried through and the samples analyzed chemically.

Determination of the proteolytic strength of the different samples, made in association with our colleagues, L. M. Gerdes and W. L. Seibert, was in accordance with the method given in the 9th revision of the U. S. Pharmacopoeia.[2] The assays were checked in each case, and controlled by running through a standard (1:3000) pepsin under identical conditions.

The chemical examination included analyses of total mineral matter, total nitrogen, total sulfur by the method of Wolf and Osterberg,[3] volumetric estimation, in the ash, of phosphoric acid as P_2O_5,[4] chlorides as NaCl,[5] calcium as CaO; also, determination of nitrogen existing in coagulable protein, proteoses by zinc sulfate precipitation,[6] peptones by Bigelow and Cook's[1] modification of Sjerning's method, and amino-acids according to Van Slyke.[2] In addition, observations were made in a 2% aqueous solution of optical rotation, and of the hydrogen-ion concentration. The direct reading ionometer described by Bartell[3] was used in the latter, with a Weston Standard Cell, and the chain: Calomel electrode (N KCl)—saturated KCl—pepsin solution—Pt electrode—H_2 at 23°. The complete "set up" employed was similar to that used by Davis[4] in a previous investigation of diphtheria toxin.

Supplementing the preceding, qualitative tests were carried out in accordance with the technique employed by one of us, Davis,[5] with peptone samples. Both a straight 2% aqueous solution and the filtrate, after coagulating the protein, were used, and examination made for: tyrosin (xanthoproteic, Millon's reaction) tryptophane (Adamkiewicz Hopkins-Cole reagent), glycoprotein and glycoproteose (Molisch reagent). Tests were

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[2] "Pharmacopoeia of the United States," 1916, p. 312, 9th Rev., P. Blakiston's Son & Co.

[3] Wolf and Osterberg, *Biochem. Z.*, 29, 429 (1910).

[4] "Methods of Analysis, A. O. A. C.," U. S. Dept. Agr., Bur. Chem., *Rev. Bull.* 107, 4 (1912).

[5] "Standard Methods of Water Analysis," *Am. Pub. Health Ass'n*, 1917, p. 4.

[6] Bömer, *Z. anal Chem.*, 5, 562 (1895).

[1] Bigelow and Cook, *J. A. Chem. Soc.*, 38, 1496 (1906).

[2] Van Slyke, *J. Biol. Chem.*, 16, 121 (1913).

[3] Bartell, *J. A. Chem. Soc.*, 39, 630 (1917).

[4] Davis, *J. Lab. Clin. Med.*, 3, 358 (1918).

[5] Davis, *Ibid.*, 3, 75 (1917).

TABLE II.—REACTIONS IN 2% SOLUTION.

Proteolytic strength. (U. S. P. IX.)	Character of solution.	Picric acid reaction.	Ammonium sulfate reaction.	Millon's reagent.	Biuret reaction.	Hopkins-Cole reagent.	Molisch's reagent.	Xanthoproteic reaction.
1:2,000	Straight			Mod. ppt., reddish	Bluish pink	Ppt., ring	Ppt., ring	Yellow color ppt., orange
	Coag. filtrate	Mod. ppt.	No. ppt.	Sl. ppt., reddish	Bluish pink	No ppt., ring	Ppt., ring	Yellow, deep orange
1:5,500	Straight			Heavy ppt., pink	Bluish pink	Ppt., ring	Ppt., ring	Ppt., orange
	Coag. filtrate	Mod. ppt.	No. ppt.	Sl. ppt., reddish	Bluish pink	No ppt., ring	Ppt., ring	No ppt., mod. orange
1:6,000	Straight			Mod. ppt., pink	Bluish pink	Ppt., ring	Ppt., ring	Ppt., mod. orange
	Coag. filtrate	Mod. ppt.	No. ppt.	Sl. ppt., pink	Bluish lavender	No ppt., ring	Ppt., ring	No ppt., deep orange
1:10,000	Straight			Heavy ppt., pink	Bluish pink	Ppt., ring	Ppt., ring	No ppt., light orange
	Coag. filtrate	Mod. ppt.	No. ppt.	Sl. ppt., pink	Bluish lavender	No ppt., ring	Ppt., ring	Ppt., orange
1:12,000	Straight			Heavy ppt., pink	Bluish pink	Ppt., ring	Ppt., ring	No ppt., light orange
	Coag. filtrate	Mod. ppt.	No. ppt.	Sl. ppt., pink	Bluish lavender	No ppt., ring	Ppt., ring	Ppt., orange
1:18,000	Straight			Heavy ppt., reddish	Bluish pink	Ppt., ring	Ppt., ring	No ppt., red-orange
	Coag. filtrate	Mod. ppt.	No. ppt.	Sl. ppt., pink	Bluish lavender	No ppt., ring	Ppt., ring	Ppt., red-orange
1:21,000	Straight			Heavy ppt., pink	Bluish pink	Ppt., ring	Ppt., ring	No ppt., orange
	Coag. filtrate	Mod. ppt.	Sl. opal	Sl. ppt., red tinge	Bluish lavender	No ppt., ring	Ppt., ring	Ppt., red-orange
1:24,000	Straight			Heavy ppt., pink	Bluish pink	Ppt., ring	Ppt., ring	No ppt., yellow-orange
	Coag. filtrate	Sl. ppt.	Sl. opal	Sl. ppt., red tinge	Bluish lavender	No ppt., ring	Ppt., ring	Ppt., red-orange
1:28,000	Straight			Heavy ppt., pink	Bluish lavender	Ppt., ring	Ppt., ring	No ppt., faint reddish
	Coag. filtrate	Sl. ppt.	Sl. opal	Trace ppt., red tinge	Bluish lavender	No ppt., trace ring	Ppt., ring	Ppt., red-orange
1:40,000	Straight			Heavy ppt., pink	Bluish ppt., lavender	Ppt., ring	Ppt., ring	No ppt., faint reddish
	Coag. filtrate	Opal	Opal	Opal red tinge	Bluish, no color	No. ppt., no ring	Ppt., ring	Ppt., deep orange

also made on the filtrate from coagulable protein, for proteoses (by addition of saturated zinc sulfate, ammonium sulfate, picric acid solutions), and protoproteoses (by saturated sodium chloride solution, potassium ferrocyanide in acetic acid solution).

RESULTS.—Altogether, nine purified products were prepared. Including the basic pepsin material, the various proteolytic strengths of the enzyme which were examined ranged from 1:2000 to 1:40,000 (U. S. P. IX). The results given in the accompanying Tables I and II are, in every case, based on duplicate determinations and, because of possible variation in the U. S. P. pepsin assay, these estimations were carried out in triplicate by two different observers.

As may be noted from Table I, the purification of pepsin is accompanied by a general decrease in the total mineral matter. This ranges from an ash content of nearly 5.5% in the case of the basic (1:2000) product down to about 2% with the highest proteolytic strengths obtained. The phosphoric acid content, also, shows a gradual decrease so that the value at 1:40,000 is less than one-third that of the basic material. Both the calcium oxide and total sulfur values fluctuate in the different strengths but both show an increase in the purified as compared with the unpurified samples. It is a significant fact that the chlorides, which are present to the extent of 1.19% (as NaCl) in the 1:2000 sample, practically disappear as a result of purification.

Probably the most important data are furnished by the various nitrogen factors, particularly the nitrogen in amino-acid condition. Confirming more elaborately the results found by Aldrich,[1] there is found to be almost a uniform decrease in *a*-amino-acid nitrogen so that in the sample testing 1:40,000 only 0.61% is found. Corroborating these results, it will be noted from the table that there are steady increases in both the coagulable protein nitrogen and that existing as proteoses, while the peptone nitrogen like that of the amino-acids shows a decrease. The values for total nitrogen showed decided variations among the different samples, with no significant change as the purification increased.

All of the different strengths of the pepsin examined show levorotation in very nearly the same degree, so that this factor is apparently unaltered as a result of purification. With the excep-

[1]Aldrich, *J. Biol. Chem.*, 23, 339 (1915).

tion of the strongest sample obtained (1:40,000) a slight amount of hydrochloric acid was used in the preparation of the other strengths of the pepsin. As a consequence, 2% aqueous solutions of these samples show relatively high hydrogen-ion concentration. However, the reaction of 1:40,000 sample, which is the nearest approach to the pure enzyme, is very nearly neutral ($C_h+ = 6.6 \times 10^{-7}$). This would tend to disprove the view held by Jacoby[2] and others that pepsin is an acid.

Consideration of the data presented in Table II shows that the results corroborate, in a general way, the analytical data already discussed. No tests were made on the straight pepsin solutions with saturated picric acid, sodium chloride and ammonium sulfate solutions, and also none with potassium ferrocyanide in acetic acid solution, since the results with all of these reagents, because of coagulable protein, would be positive, and practically the same for the different strengths. Confirming the results given in Table I, the saturated picric acid, Hopkins-Cole, and Millon's reagent tests, made of the filtrate after removal of coagulable protein, show presence of amino-acid and peptid bodies in the lower strength samples. These gradually disappear so that only traces are found in the highest strength sample of the enzyme. Both saturated sodium chloride solution and potassium ferrocyanide in acetic acid solution gave negative results, indicating absence of protoproteoses in the filtrate from coagulable protein. A positive reaction was obtained in every case with Molisch reagent showing presence of glycoprotein, or its derivatives, in the material. It is significant that the biuret test of the filtrate after coagulation of protein in the 1:40,000 sample is negative. This would indicate that practically all of the protein material is of the nature of coagulable protein or even more complex in its protein character.

DISCUSSION

A review of the data presented in the foregoing seems to show that in the purification of pepsin there is a gradual elimination of the secondary protein derivatives, including amino-acids. This is manifested by a constant tendency in the purified samples to ap-

[2] Jacoby, *Biochem. Z.*, 4, 471 (1907).

proach nearer to the actual character of proteins with increasing proteolytic activity, accompanied by an increase in material coagulable by heat. From the fact that the highest strength samples still give strong tests with Molisch reagent, it may be possible that the pure enzyme is a conjugated protein, probably a glycoprotein.

Confirming this view, the mineral matter is decidedly less in the purified samples than in the original basic material, approaching almost to the value for pure proteins in the case of the strongest samples. Both sulfur and calcium are probably unaffected by the purification, but there is a decided decrease in the phosphorus content and seemingly a total elimination of chlorides. Other than the increase which would obtain by removal of non-nitrogenous impurities, there is probably not much change in the content of total nitrogen as a result of pepsin purification.

The manner in which the *a*-amino-acids decrease as the proteolytic activity increases is striking, and seems to be almost proportional in amount. It is noteworthy that the small amount of *a*-amino-acid present in the sample testing 1:40,000 (0.61%) very nearly approaches the value for this factor due to lysin as found present by Van Slyke and Birchard[1] in most proteins analyzed by the nitrous acid method.

Results of optical activity determinations are apparently of no significance, since the same values are obtained with several different strengths of pepsin. As already mentioned above, the reaction in aqueous solution of the strongest (1:40,000) pepsin is significant because of its very slight acidity. It would seem very likely that the concentration of hydrogen-ions in solutions of the pure enzyme, when isolated, will probably show only the slight acidity comparable to that given by other proteins.

In connection with the assays of proteolytic strength by the U. S. P. method, it was deemed of interest to make a comparison of the rennetic power of the different samples. It is a significant fact that throughout the entire series, from 1:2000 to 1:40,000, the rennetic activity and proteolytic strengths are found to go hand in hand. This is being investigated, and will be reported upon in a later paper.

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[1] Van Slyke and Birchard, *J. Biol. Chem.*, 16, 539 (1914).

CONCLUSIONS

1. The purification of pepsin seems to consist in the elimination of secondary protein derivatives, including a-amino-acids.

2. Calcium and sulfur appear to be unaltered as a result of purification, but phosphorus is materially reduced. Chlorides are seemingly entirely removed.

3. Aqueous solutions of pepsin, after purification, show no material change in optical activity. A sample of high digestive power (1:40,000) shows a reaction very nearly neutral.

4. Pepsin tends to approach nearer to the actual character of a protein (possibly a glycoprotein) with increasing proteolytic activity.

**Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 198, 1919.**

(Reprinted from the *Journal of the American Veterinary Medical Association,* Vol. LIV, N. S. Vol. 7, No. 7, March, 1919.)

THE PRESENT STATUS OF SPECIFIC TREATMENT FOR CONTAGIOUS ABORTION.*

H. Preston Hoskins, V. M. D.

(From the Medical Research Laboratories of Parke, Davis & Co., Detroit, Mich.)

It is now over twenty years since Bang[1] announced the discovery of the causative organism of contagious abortion of cattle. The identity of the organism responsible for the same disease in this country, with that of the European organism, was established by MacNeal and Kerr[2], in Illinois, in 1910. These discoveries stimulated many investigators to attack the problem of finding some remedy or agent for the prevention or cure of the disease. Naturally the search for such an agent was in two directions, viz., medicinal and biologic. This paper will deal only with the latter, although it might be stated, in passing, that up to the present time anything in the way of a chemo-therapeutic agent of real merit is much of the nature of a chimera.

The fact that the behavior of contagious abortion in a given herd, when not interfered with by hand of man, suggests that a rather high degree of immunity is established after a succession of abortions, has led to experiments to produce such an immunity artificially. The agents used have been products prepared from cultures of the abortion organism, almost without exception. Most of the work on which reports are available has been done with killed culture of B. abortus, so-called abortion bacterins and vaccines.

Various methods have been used in the preparation of these vaccines. The dosages recommended by the purveyors of these products show extreme variations, from what appears to be a rather low dosage to what is considered a high dosage (from 85 billion to 1000 billion organisms). The number of injections

*Read before the Southeastern Michigan Veterinary Medical Association, Detroit, Michigan, January 8, 1919.

advised is equally inconsistent, some advocating three, some four, and some six treatments. The intervals between injections show the same wide variations. In fact, the practitioner can at present choose just about what he wants to suit his own convenience in the matter, or his preferences, if he has any. If he has to take into consideration the size of his bill, or his client's pocketbook, he can select anything from a course of treatment costing 75 cents to one costing $2.00 per animal. To the best of our knowledge there is no publication available which gives directions for varying the dosage or the intervals between injections to meet local or individual conditions. Further, it might very properly be argued that the very reason why there is such a wide variation in the matter of recommended dosages is because none of the dosages employed has given uniformly good results.

At the recent meeting of the United States Live Stock Sanitary Association, held in Chicago in December, Dr. Williams very severely criticized certain biological manufacturers for continuing to market contagious abortion vaccines, in the face of the practical unanimity of opinion of competnet authorities to the effect that such products were just about worthless. "No-cure-no-pay" propositions of various concerns came in for their share of censure at the hands of Dr. Williams, as well as the misleading advertisements of others. A great deal of credit was and should be given Dr. Williams for his courage in taking the stand he did. The evidence available certainly justifies his attitude.

For the purpose of getting the consensus of expressed opinion of various authorities on the value of contagious abortion vaccines, the writer has examined all recent reports and publications available. Care has been used to select only the most recent of these papers and reports for abstracting opinion, so that the résumé can be said to consist only of the very latest and up-to-date ideas on the subject. Besides this evidence, an opportunity has been afforded to examine a large number of reports on the use of contagious abortion vaccine experimentally. Many of these reports are valueless from a truly scientific standpoint, owing to the lack of care used in keeping accurate records and making final reports.

It is only fair to state that in quite a number of cases the outcome of the treatment was reported as "favorable" or "satis-

factory," in the judgment of the owner or the attending veterinarian. Except in rare cases, no cows were left untreated as controls in the experiments reported. Equally rare was a complete herd history. In many instances all the animals in certain herds were treated alike, virgin heifers, heifers in calf for the first time, mature cows, suspected aborters, known aborters, nonbreeders, etc. Even under the best of conditions it is not often possible to get complete data upon which to base a fair estimate of the results of the treatment. Such cases do occur, however.

To show how easy it is for one to be led to believe he is getting desired results from a certain treatment, an instance will be cited which is illustrative, and at the same time rather amusing. A veterinarian wrote in to obtain some contagious abortion vaccine. The requisite number of treatments of our experimental product was forwarded. The vaccine was for bovines and so labeled. In due course of time a report on the outcome of the treatment was solicited. We were informed that the vaccine had been used on eleven mares, no abortions followed, and naturally the vaccine received credit for 100% success. Although this might be used as an example of non-specific therapy, we rather incline to the belief that it was a case where nature was not given the credit due her, but was overlooked because the vaccine had been used. Salt solution would probably have been just as efficacious in this particular case.

Some veterinarians deserve to be censured for the attitude they take on this question. Fortunately, known cases such as the following are few, but they do exist nevertheless, and should be frowned upon whenever encountered, certainly not encouraged. Dr. A. is asked to advise a client how to proceed to rid his herd of contagious abortion. He elicits from his client the information that the disease has existed in the herd for a year or more. Dr. A. has used contagious abortion vaccines in several herds with questionable results. At the same time he is aware of the irregular results reported by fellow practitioners. However, he advises his client that vaccination is indicated. When asked why he adopted this course, his explanation is somewhat as follows:

"Well, in the first place, I had to recommend something. From the history of the herd, I was of the opinion that the worst was over, and that probably there would not be many more abor-

tions. I did not advise vaccination because I thought vaccine would clean up the herd. Between you and me, I don't believe the stuff is any good, anyhow. But I knew that if I did not vaccinate that man's herd, Dr. B. would, and there you are. I had to do something."

Manufacturers have been accused of making false claims for contagious abortion vaccines, thereby creating a demand for them, and, having created this demand, they feel fully justified in meeting it, very much for the same reason that Dr. A. treated his client's herd. Manufacturer X says that if he does not sell Dr. A. contagious abortion vaccine, his competitor will. In the case of this particular product it cannot be said that the demand comes as a result of the publication of work indicating that the product is valuable or has merit. At the present time the line is pretty sharply defined between those who publicly support the vaccine method of treatment and those who do not. On the one side we have those whose vision is dimmed by commercialism, while, on the other, we have men who have spent years in investigating and observing, and are in position to give opinions that are free from bias and prejudice. These opinions are numerous and come from some of the very best men in the veterinary profession today.

There are a number of points that must not be lost sight of, if we wish to give a fair interpretation of the results obtained following the use of contagious abortion vaccine.

1. In the great majority of cases the vaccine is resorted to only in the declining phase of the trouble in a given herd.

2. Abortion disease is frequently a complicated pathological condition, and the number of abortions in a herd does not always tell the whole story.

3. Undoubtedly there are local conditions which play a prominent part in lessening or increasing the severity of the disease, regardless of artificial interference, and for this reason it is difficult to evaluate properly the results obtained by our present hit-or-miss, go-as-you-please methods.

Some rather questionable statements are found in the advertising matter distributed by certain laboratories. Frequently such statements are not accompanied by any experimental data to substantiate them, nor any references to such data. As an

example, we will quote a paragraph from a circular on the subject of anti-abortion vaccine, viz: "The British commission reported that in instances where live cultures were used, the results were successful, but were of little or no avail if cultures were heated. Anti-abortion vaccine is prepared from a large number of strains of the bacillus of infectious abortion, isolated from widely scattered sections of America. *The organisms are killed without the aid of heat, which makes them practically as beneficial in producing immunity as though live cultures were used.*" I seriously question this statement.

Reference has been made to the opinions expressed by competent authorities on the value of vaccines. The annual report of the State Veterinarian of Nebraska[3], for the year 1918, contains the following general statement concerning contagious abortion and sterility, and the remarks on vaccine treatment are illustrative of the present trend of opinion:

"The way to guard against the disease is by prevention and sanitation. Be careful when buying new animals to be placed in the herd. There are no drugs which are of any value in the treatment of this disease except antiseptics. Carbolic acid, methylene blue, and some other drugs, which some claim have proven useful, are, in fact, of no value whatever. Anti-abortion bacterins have been put on the market and are being advertised extensively, but the manufacturers have as yet not been able to prove their value. Excepting the claims made by the biological houses, which they seem to be neither able nor willing to substantiate, there is no evidence whatever to prove that anti-abortion bacterins are worth anything. The best that can be said about them is that they are still in the experimental stage. This does not mean that they will never be perfected to a higher degree of usefulness, such as other biological preparations have been developed, but until such time it would be folly to depend on their use in the prevention of the disease, and it would be money wasted to buy them."

A recent bulletin from the North Dakota Agricultural Experiment Station[4], on the subject of abortion disease in cattle, contains the following paragraph:

"The fact that after abortion a certain degree of immunity is apparently established, combined with the success acquired through artificial immunity in a series of other diseases, is re-

sponsible for various attempts to control abortion disease by rendering the animals refractory to it by similar methods. In this country very little work has been done in this direction, partly, no doubt, because until the last few years the seriousness of the disease was not generally recognized. A far greater alacrity, however, was exhibited by the commercial interests who for some time already had flooded the market with a great number of so-called bacterins or supposed immunizing agents, claimed to have a great value in the prevention of a considerable assortment of diseases. The opportunity offered by the now well-advertised abortion disease was not wasted by those interests, and bacterins are now on sale, accompanied by even more or less spurious 'guarantees.' Those and similar bacterins consist of suspensions of killed bacteria and hence they are probably entirely harmless. Their usefulness may, however, be questioned in the absence of well-controlled experiments and observations."

McFadyean and Stockman[5], in commenting upon the results of experiments with "bacterin" treatment, state that the influence of the killed cultures was practically negligible, and their published data certainly bear out this conclusion.

In a paper by Drs. Adolph Eichhorn and George M. Potter[6], read before the American Veterinary Medical Association, in this city, August 22, 1916, and published in the Journal of the Association, the following reference is made to the use of bacterins:

"Immunization with abortion bacterins is now being widely advocated by manufacturers of these products. The results obtained do not warrant the confidence which is expressed in the literature and advertising matter. Considerable experimental work has been conducted by the Bureau of Animal Industry on the effectiveness of bacterin treatment, and, while the results were somewhat encouraging, nevertheless generally good results cannot be claimed for such a procedure. In view of our findings, and also those of other investigators, the claims for the bacterins are unwarranted, and will not serve any good purpose in the control of the disease. Veterinarians will be prone to accept the statements made by the manufacturers at their full value, and possibly disregard other effective means by which the disease might be combated. It is possible that further investigations will establish a more effective method of immunization, but at present the bacterin treatment should be regarded as second in importance to proper sanitation."

Speaking from the standpoint of a practitioner, Dr. Cotton[7] states as follows:

"Until we have something more positive in the line of immunization by vaccination, I am of the opinion that we, as practitioners, should not undertake to build up false hopes in the minds of owners of our herds throughout the country, by the use of the various vaccines that are now on the market."

In an article treating of abortion and its sequelæ, Dr. C. C. Palmer[8] of Delaware College speaks of his experiences as follows:

"We have tried a system of vaccinating (by means of serobacterin) all new-born calves, but the results so far have been disappointing. Many of the calves seem to do well until two or three months of age, when they invariably develop scours and pneumonia. Further treatment with vaccines or serum was unavailing.

"Biological products for the control of calf diseases should be regarded as being in the experimental stage, and at the present time our chief hope lies in the proper hygiene of these young animals. Vaccines and serums may be tried, but the hygienic factor should not be lost sight of."

I am personally familiar with the work that has been done by Dr. W. L. Boyd, at the University of Minnesota, and the following paragraph from the 25th annual report of the Agricultural Experiment Station, University of Minnesota[9], is merely an illustration of the results obtained in large numbers of experiments:

"Thirty-two young heifers were given a series of injections of large doses of killed abortion bacilli. The results so far obtained indicate that this method of vaccination is not satisfactory for the prevention and control of infectious abortion."

The opinions of Dr. Williams on this subject are so well known that they need little comment. However, in a recent publication[10] he states:

"The administration of bacterins, vaccines or sera to the pregnant animals has been advocated, but these have failed. Like antiseptics, they fail to reach the location of the harmful infections in the uterine cavity. Their failure does not prevent some establishments from advertising and selling their products."

It should be distinctly understood that practically all of this adverse criticism has been directed against contagious abortion bacterins and vaccines consisting of dead organisms, whether they be killed with or without the application of heat. The use of vaccines containing living organisms is being turned to just now, in the hope that they will give a better account of themselves. The work of the English Commission and of Bang, in Denmark, is referred to in this connection. Experiments on a small scale are now being conducted in this country, and the results will be awaited with interest. It will likewise be of interest to note what the attitude of sanitary officials will be as to the distribution and use of the living vaccine. That it is a procedure attended by a certain amount of danger goes without saying.

The disadvantages of employing live bacilli are enumerated in a recent bulletin prepared by Dr. F. B. Hadley[11] of the Wisconsin Agricultural Experiment Station. They are as follows:

(1) Rapid deterioration of the vaccine.

(2) Slow development of immunity.

(3) Temporary discomfort to the animal.

(4) Danger of introducing the infection.

Of these objections, the second, the slow development of immunity, will probably be the most difficult to overcome. This is no fault of the vaccine, but is due to the very nature of the disease. It is always a more or less chronic infection. Practically without exception, those diseases which are successfully controlled by vaccination are the acute infections, such as blackleg, hog cholera, anthrax, hemorrhagic septicemia, and rinderpest, while such chronic diseases as tuberculosis and glanders have not been amenable to prophylaxis by any system of vaccination.

When we speak of immunity in contagious abortion it is not exactly clear what we mean by the term. Our laboratory tests that indicate a positive reaction tell us little of value in the case of the individual animal. An animal reacting positively to one of these tests today may abort tomorrow, or she may carry her calf to full term, and subsequent pregnancies may be terminated just as successfully. The future of these calves, however, is another matter.

This paper would not be complete without some mention of the use of a specific anti-serum. Some experimental work has

been done with this product, but, again, the nature of the disease is such that the extensive use of the serum may never come about. When used as a prophylactic, the immunity afforded probably does not last over three weeks, necessitating a repetition of the treatment every three weeks until pregnancy is terminated. This method of treatment is too expensive, except in the case of extremely valuable animals.

As a curative agent the serum is again of little use, as far as the cow is concerned, on account of the nature of the disease. The abortion organism is rarely the cause of the death of the cow. If there are any complications following an abortion they are usually not due to the abortion organism, and therefore an abortion anti-serum is not indicated. Investigations would seem to indicate that the abortion organism is responsible for some of the common calf diseases, notably white scours, calf pneumonia, arthritis (navel-ill), etc. Therefore, a serum directed against this organism is indicated. However, there are other organisms which are just as likely to be implicated in these conditions, most important of which is the colon bacillus.

It is the practice of some laboratories to include the abortion organism in the antigen used for the production of white scours serum. We carried this idea even further and prepared a serum using equal amounts of abortion and colon organisms in the antigen. This was used on herds where both white scours and contagious abortion existed. Clinical reports indicated that the results obtained with this combination serum were no better than when the regular white scours serum was used, and we decided to let well enough alone.

In closing, I would like to call your attention to a recent publication on the subject of the etiology of contagious abortion of cattle. If the findings of Dr. Theobald Smith[12] of the Rockefeller Institute, Department of Animal Pathology, are verified by other workers in different parts of the United States the least that can be said is that the question is more complicated than ever.

Dr. Smith has recently described an organism, a spirillum, which he has isolated in pure culture from the fœtuses of fourteen cases of abortion. The abortions occurred in a group of herds, under the same management, and during the investigation, which lasted over a period of about fifteen months, there were twenty-

seven abortions, in which B. abortus of Bang appeared to be the etiological factor.

One of the most interesting facts brought out by the investigation is the absence of B. abortus in those cases where the spirillum was found and the absence of the latter when B. abortus was present. That the two organisms are not identical is shown by the lack of pathogenicity for guinea-pigs, on the part of the spirillum, besides difference in morphology.

On the other hand, there are a number of points in common. The spirillum is evidently an organism that demands a reduced oxygen tension for successful artificial cultivation, and it has been found that it may be isolated in pure cultures from fœtuses under precisely the same conditions as B. abortus. Both organisms have been found in the gastro-intestinal tract, as well as the respiratory tract of the fœtus, quite regularly, but less frequently in the other organs. Both organisms have been found in the placental fluids.

For some unexplained reason the spirillum has not caused an abortion during the first pregnancy of any cow, but in all cases in which the spirillum has thus far been demonstrated the abortion has occurred during a second or later pregnancy. No inoculation tests have yet been made owing to a lack of suitable subjects. It is desirable—in fact, essential—that experiment animals used in the crucial test should be perfectly free from infection, with either Bang's bacillus or the spirillum, and they should likewise be free from any immunity to these infections gained by exposure to them. Serological tests have been delayed, owing to the difficulty of getting the organism to grow satisfactorily on artificial media not containing blood or tissues.

If subsequent investigations show that the new organism is capable of producing abortion, as does the Bang bacillus, it is difficult to predict just what effect it will have on our present methods of handling the abortion problem. The next step will be for other investigators, in different localities, to try and confirm the findings of Dr. Smith. The fact that abortions among sheep and cattle in Ireland and Wales have already been reported as due to organisms similar to the spirillum is quite suggestive of the widespread character of the infection. Are the irregular results that have been obtained with bacterins made from B.

abortus due to the fact that this organism was not operating in a certain percentage of the cases?

CONCLUSIONS.

1. The consensus of opinion, as expressed by recognized authorities, is to the effect that the vaccines and bacterins made from dead abortion bacilli are of no real value in the prevention, cure or control of the disease.

2. Such preparations are probably harmless, in so far as any danger to the treated animals is concerned, but they may possess a potential danger by causing veterinarians to overlook other methods for handling the disease.

3. If veterinarians wish to make and preserve a reputation for being careful observers, scientific workers, and ethical practitioners, they should keep in mind the nature of this abortion disease and its sequelæ, treat it accordingly and endeavor to interpret results intelligently, at all times holding themselves aloof from any connection with the so-called no-cure-no-pay propositions.

4. Experiments with vaccines consisting of living organisms are apparently more encouraging, according to the work thus far reported. If a stable product can be prepared, for use under actual conditions, and a safe method of distribution and administration devised, the product may prove to be of considerable value in the control of the disease.

5. The use of anti-abortion serum is rather limited, owing to the nature of the disease. Theoretically, its employment in certain conditions is perfectly rational, if the value of the cow or her progeny warrants the expense.

6. Some clinical observations would suggest that the colon organism is more important in the common diseases of the new-born calf than B. abortus, even though the latter is found in some cases of scours, pneumonia, arthritis, etc.

7. Our problem may be further complicated if the researches of Dr. Theobald Smith are confirmed, and it is shown that there are two organisms, instead of one, responsible for the abortions so prevalent among our cattle.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 199, 1919.

(Reprinted from the *Journal of the American Veterinary Medical Association*, Vol. LV, N. S. Vol. 8, No. 1, April, 1919.)

PRACTICAL METHODS OF TREATMENT FOR WORM INFESTATION.*

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This paper will deal as briefly as possible with a dozen of the more important worm parasites affecting horses, cattle, sheep, swine, dogs, cats and poultry. It will approve the use of perhaps a half-dozen drugs. It will deal with nematode worms primarily. In the belief of the writer, this is a practical paper. There is a disposition among practitioners to regard laboratory men as impractical. As a laboratory man, it is the writer's belief that as regards the use of anthelmintics the practitioner is frequently highly impractical in that he often uses inefficient measures in place of equally feasible effective ones. The determination of the value of anthelmintic medication in practice is not always a simple and certain procedure. Under what we may call barnyard, stable, hog-pen and kennel conditions, it is not always easy to ascertain what worms are passed. There are too many complications. The manure of one animal is mixed with that of other animals, and that of one day with that of other days. The farmer or the stable hand is commonly uncertain as to just what has happened and neither their observation nor their judgment can be trusted in many cases. The technique of examination necessary under such circumstances is crude and unsatisfactory. Poking in the manure with a stick gives a minimum amount of information. The number of worms passed may sometimes be ascertained or guessed at, if the worms happen to be large ones, but the number left cannot be readily ascertained and clinical evidence of recovery from a sub-chronic afebrile state of malnutrition and impoverishment, such as is commonly present in

*Presented at Section on Sanitary Science and Police, 55th Annual Meeting, A. V. M. A., Philadelphia, 1918.

clinical cases of parasitism, comes slowly, and such evidence can seldom be sought for by the busy practitioner. Under these circumstances, drugs of but little anthelmintic value became established as anthelmintics. Strictly speaking, treatment with such drugs is not practical treatment.

These are conditions practically inseparable from the practice of veterinary medicine and no criticism can be fairly leveled at the practitioner on that score. What we need in this case are dependable anthelmintics, with an efficiency established by numerous tests under experimental conditions. This is the task of the laboratory man. It is a task that has been undertaken, but only a small part of the work has yet been done. Part of what I may say today will probably prove to be premature in the light of what we will know next year, but if we waited till we knew everything about a subject before telling it, rather than tell the little we know, our progress might be surer, but it would undoubtedly be much slower.

Critical laboratory tests of numerous reputed anthelmintics, and there are hundreds of substances that have had anthelmintic value claimed for them, show that most of the anthelmintics have less value, often much less, than is commonly assigned to them, while a few prove on critical test to have the value which clinical evidence has attributed to them. These results naturally lead to skepticism in regard to the actual value of untested anthelmintics. By a critical test I mean the administration of the drug to experiment animals under fixed conditions, the subsequent collection of all feces passed, the careful collection of worms from such feces daily for a period of four or more days, and, finally, the killing of the experiment animal at the end of the experiment and the post-mortem collection of all parasites not removed in order to ascertain where the treatment failed, as well as where it succeeded. Most of the recommendations I wish to make are based on such tests, and where they are not, I make my recommendations with reservations.

The horse is infested with a number of worms, of which we will consider only the strongyles, pinworms and ascarids.

The strongyles of the horse include several genera, but the two genera of importance in the large intestine are the genus *Strongylus*, sometimes called *Sclerostomum* (the large red pali-

sade worms), and the genus *Cylicostomum*, also called *Cylichnostomum* and *Trichonema* (the small worm from the large intestine). The latter genus includes a rather large number of species which have been lumped under the name *Strongylus tetracanthus* or *Sclerostomum tetracanthum*. There are 3 species of *Strongylus* commonly present in horses, *Strongylus equinus*, *S. edentatus* and *S. vulgaris*. Species of both genera are commonly present in the same animal and often in large numbers. The disease due to the presence of the strongyles of both genera has been called strongylidosis by Leneveu. It is an afebrile, wasting disease, characterized by digestive disorders, debility, anemia and edema, and complicated by the many serious sequelæ resulting from aneurism production by *S. vulgaris*.

The importance of our horses for cavalry, field artillery and transport purposes at this time, the value of these horses by the time they arrive in France, and the prevalence of strongylidosis make the subject of treatment for this disease a matter well worthy of our consideration.

It is generally understood that these worms are hard to remove, and on that point the veterinary practitioner and the parasitologist are in agreement. It is, therefore, somewhat surprising to learn that, on the contrary, these worms are not particularly difficult to remove. In tests which I made in collaboration with Dr. R. H. Wilson and Mr. Meyer Wigdor, we obtained very high anthelmintic efficacy in the treatment of strongylidosis. The results of our experiments are detailed in a paper which is already in press, but I will give a synopsis of those results here.

We secured the removal of every *Strongylus*, 107 of them, from a horse that had fasted over 24 hours, by the administration of 16 mils of oil of chenopodium, followed immediately by a quart of linseed oil. We removed 96 per cent of the *Strongylus* present, 66 out of 69, from a horse that had fasted over 24 hours, by the administration of 16 mils of oil of chenopodium, followed 2 hours later by a quart of linseed oil. We removed 95 per cent of the *Strongylus* present, 78 out of 82, from a horse that had been fasted over 24 hours, by the administration of 3 6-mil doses of oil of chenopodium at hour intervals, followed an hour after the last dose by a liter of linseed oil. We removed 76 per cent

of the *Strongylus* present, 61 out of 80, from a horse that had been fasted less than 24 hours, by the administration of 16 mils of oil of chenopodium, followed immediately by a quart of linseed oil. We removed 48 per cent of the *Strongylus* present, 102 out of 214, from a horse that had been fasted less than 24 hours, by the administration of 2 ounces of turpentine in a quart of linseed oil. We removed less than 1 per cent of the *Strongylus* present from one horse that received 18 mils of oil of chenopodium and a quart of linseed oil after fasting less than 24 hours; from a second horse that received 18 mils of oil of chenopodium and a quart of linseed oil after fasting less than 24 hours; from a third horse that received 8 mils of oil of chenopodium and a quart of linseed oil after fasting 24 hours; from a fourth horse that received 2 drams of tartar emetic in a mash daily for 5 days; and from a fifth horse that received 2 drams of iron sulphate in a mash daily for 7 days. The foregoing shows that 96 and 100 per cent efficacy were secured by the use of 16 mils of oil of chenopodium, followed immediately or 2 hours later by a quart of linseed oil, in animals that had been fasted over 24 hours. In these cases, the horses were given a light feed in the evening and all hay and bedding removed. No food was given the next day. The next day the treatment was given early in the morning and the animal not fed for 3 hours afterward. Where animals were fed the morning before treatment, equally good results were not obtained.

With approximately the same size of dose, the efficacy fell to 76 per cent in one case and less than 1 per cent in another. With smaller doses, 10 mils and 8 mils, the efficacy remained below 1 per cent. It would appear from this that doses of about 16 mils of oil of chenopodium were needed and that the animal should be fasted over a period of almost 36 hours to insure the best results. The one experiment with turpentine, securing in 2-ounce dose in a quart of linseed oil the removal of 48 per cent of the *Strongylus* present in a horse fasted for less than 24 hours, indicates that we have in turpentine a fairly effective substitute for oil of chenopodium when the latter is unobtainable.

Some experiments I performed previous to the experiments noted above bear out our findings in a general way. Horse No. 1 was given 8 mils of oil of chenopodium, followed immediately

by a liter of linseed oil, after fasting for less than 24 hours. The treatment removed 51 per cent of the *Strongylus* present, 19 out of 37. Horse No. 2 was given 16 mils of oil of chenopodium, followed immediately by 800 mils of linseed oil, and 100 mils of castor oil, after fasting for 24 hours. The treatment failed to remove any *Strongylus*. Fifteen days later this horse was given 20 mils of choloroform, followed in 15 minutes by 750 mils of linseed oil. The animal had been given some feed shortly before treatment. This treatment also failed to remove any *Strongylus*. The animal was killed 6 days later and found to have 3 *Strongylus* in the cecum. The explanation for the failure of treatment here probably lies in the presence of a very small number of *Strongylus*, perhaps safe in a remote part of the cecum. Horse No. 3 was given 12 mils of oil of chenopodium, followed by 800 mils of linseed oil, after fasting for 24 hours. The treatment failed to remove any *Strongylus*. Six days later the horse was given 2 doses of 20 mils each of carbon bisulphide at a 2-hour interval, followed by 800 mils of linseed oil two and a half hours later. This treatment failed to remove any *Strongylus*. Eight days later the horse was given 3 doses of carbon bisulphide, 3 drams to the dose, at hour intervals. Sixteen days later the animal was killed and found to have 12 *Strongylus* in the cecum. The failure of the treatment here may have been due to the size of the dose, the fasting period, the presence of a few *Strongylus* in a remote portion of the cecum, or to the development of sexually mature forms from agamic worms attaining the intestine between the time of treatment and the time of death. Horse No. 4 was given 20 mils of carbon bisulphide, followed by 800 mils of castor oil one and a half hours later. The treatment failed to remove any *Strongylus*. Five days later the horse was given 12 mils of oil of chenopodium, followed immediately by 800 mils of linseed oil. The horse passed one *Strongylus*. Seven days later the horse was given 3 doses of carbon bisulphide, of 3 drams each, at hour intervals. No worms were passed and the animal was killed 10 days later. There were 13 *Strongylus* postmortem, so the treatment with chenopodium was 7 per cent effective and those with carbon bisulphide entire failures.

So far as conclusions may be drawn from the 14 experiments noted here, and we must draw what conclusions we may, since

this represents almost the entire body of dependable tests available to date, we may say that apparently *Strongylus* may be removed from horses with a rather high degree of certainty with doses of 16 to 18 mils of oil of chenopodium, followed immediately or after an interval by a quart or more of linseed oil, provided the animals have been fasted for a period of 36 hours. It would perhaps be good practice to repeat the treatment at an interval of 2 weeks.

The removal of *Cylicostomum* was even more readily accomplished in our experiments. We secured the removal of every *Cylicostomum* from 4 horses. In one of these cases the horse fasted less than 24 hours, received 16 mils of oil of chenopodium, followed immediately by a quart of linseed oil, and passed 70 *Cylicostomum*; in another case the horse fasted over 24 hours, received 16 mils of oil of chenopodium, followed 2 hours later by a quart of linseed oil, and passed 540 *Cylicostomum*; in the third case the horse fasted over 24 hours, received 3 6-mil doses of oil of chenopodium at hour intervals, followed an hour after the last dose by a liter of linseed oil, and passed 1,242 *Cylicostomum;* in the fourth case the horse fasted less than 24 hours, received 2 ounces of turpentine in a quart of linseed oil, and passed 274 *Cylicostomum*. We removed 97 per cent of the *Cylicostomum* present (or perhaps 100 per cent, if these larval forms had issued from their cysts in the mucosa after the passage of the anthelmintic, as seems likely), 77 out of 79, or 77 out of 77, as the case may be, from a horse that had been fasted over 24 hours, by the administration of 16 mils of oil of chenopodium, followed immediately by a quart of linseed oil. We removed 29 per cent of the *Cylicostomum* present, 187 out of 635, from a horse that had been fed shortly before treatment, by the administration of 10 mils of oil of chenopodium, followed immediately by a quart of linseed oil. We removed 11 per cent of the *Cylicostomum* present, 428 out of 3,623, from a horse that had been fasted less than 24 hours, by the administration of 18 mils of oil of chenopodium, followed immediately by a quart of linseed oil. We removed less than 1 per cent of the *Cylicostomum* present from 1 horse that received 8 mils of oil of chenopodium, followed immediately by a quart of linseed oil, after fasting less than 24 hours; from a second horse that received 2 drams of tartar emetic in a mash

daily for 4 days; and from a third horse that received 2 drams of iron sulphate in a mash daily for 7 days.

From the foregoing it may be concluded that in amounts of 16 to 18 mils oil of chenopodium in 1 to 3 doses, followed immediately or at an interval by linseed oil, may be expected to remove all or nearly all strongyles from the cecum and colon of the horse in many cases, provided the animal is fasted 36 hours previous to treatment.

Infestation with *Oxyuris equi*, or pinworm, is not an uncommon condition in horses, and may be suspected when horses are seen rubbing the tail against some object or when such a practice is indicated by the presence of a bare spot where the hair has been rubbed off the tail near its roots. The presence of these worms may also be suspected when yellowish egg masses are found around the anus, as a result of the crushing of the gravid female by the anal sphincter. In a recent paper, the eminent French parasitologist, Railliet (1917) states that this worm is readily removed. He notes the use of internal medication by mouth, but believes that the easy way to remove pinworms from the horse is by means of copious enemata of warm, soapy water or vinegar water, to which may be added corrosive sublimate to make a 1:2000 solution, or a mucilaginous emulsion of thymol, the enemata to be repeated as often as necessary. The treatments noted by Railliet as used in oral medication are as follows: Tartar emetic in doses of 15 to 20 grams in food; corrosive sublimate in doses of a deciliter of a 1:1000 solution daily in drink or food for about 15 days; areca nut, freshly ground, in 100-gram doses; and thymol, in 15- or 20-gram doses, suspended in mucilage.

In our experience, the removal of pinworms by means of oral medication was very easily accomplished, as a rule. In the tests in collaboration with Wilson and Wigdor, we removed 100 per cent of the pinworms present in 5 horses by the following treatments: Oil of chenopodium, 16 mils, followed immediately by a quart of linseed oil, the horse being fasted for over 24 hours; oil of chenopodium, 16 mils, followed 2 hours later by a quart of linseed oil, the horse being fasted over 24 hours; oil of chenopodium, 3 6-mil doses at hour intervals, followed an hour after the last dose by a liter of linseed oil, the horse being fasted for

over 24 hours; turpentine, 2 ounces, followed immediately by a quart of linseed oil, the horse being fasted less than 24 hours; and by tartar emetic, 2 drams daily in the feed for 5 days. We failed entirely to remove the few pinworms present in 2 cases. In one of these cases the horse received 18 mils of chenopodium, followed immediately by a quart of linseed oil, the horse being fasted less than 24 hours; in the other case the horse received 2 drams of iron sulphate daily in the feed for 7 days.

The above experiments confirm the idea expressed by Railliet as to the readiness with which pinworms may be removed. They may be cleaned out by oil of chenopodium in 16-mil doses, followed immediately or after an interval by a quart of linseed oil, in horses that have been fasted over 24 hours; by 2 ounces of turpentine, followed immediately by a quart of linseed oil in horses that have fasted less than 24 hours; or by 2-dram doses of tartar emetic in the feed daily for 5 days. So far as we can judge from so few experiments, fasting less than 24 hours interferes with the efficacy of oil of chenopodium against these worms, and iron sulphate in 2-dram doses daily for 7 days is unsatisfactory.

The writer has been under the impression for some years that the removal of ascarids, the large maw-worms, of the horse should present no special difficulties, since ascarids in man, dogs and swine yield so readily to treatment. However, test of treatments have led to the conclusion that Neveau-Lemaire (1912) was quite right in stating that none of the numerous treatments commonly employed, such as tartar emetic, turpentine, santonica, empyreumatic oil, and benzine, give satisfactory results. We failed to remove any ascarids from 4 infested animals treated as follows: One horse received 8 mils of oil of chenopodium, another 10 mils, and a third 16 mils, the chenopodium being followed immediately by a quart of linseed oil; the fourth horse received 2 ounces of turpentine, followed immediately by a quart of linseed oil. All of these horses were fasted less than 24 hours. A horse which received 2 drams of tartar emetic in feed daily for 5 days passed 8 per cent of its ascarids; one that received 16 mils of oil of chenopodium, followed immediately by a quart of linseed oil, the horse being fasted over 24 hours, passed 3 per cent of its ascarids; one that received 18 mils of oil of chenopodium, followed immediately by a quart of linseed oil, the horse being fasted less than

24 hours, passed 12 per cent of its ascarids; and one that received 3 6-mil doses of oil of chenopodium, followed an hour after the last dose by a liter of linseed oil, the horse being fasted over 24 hours, passed 25 per cent of its ascarids.

In view of the above results, the writer is unable to make recommendations in regard to treatment for ascarids in the horse. Numerous treatments are known to me, and are said to be effective. Doubtless some of these treatments are effective at least at times, but until their value has been experimentally demonstrated I would feel no confidence in them personally and would prefer to suspend judgment on this topic pending further investigation. Chenopodium is specifically ascaricidal in man, dogs and swine, and has a very high anthelmintic value. Its low efficacy against ascarids in the horse, in the dose used and in the way it was given, was a surprise. It is possible that a variation in dosage or mode of administration, such as giving a larger number of fractional doses over a longer period, may secure satisfactory results. I have found such consideration necessary in securing a satisfactory chenopodium treatment for hookworm in the dog, but given such consideration a satisfactory treatment is possible. Railliet (1915) says the preference among practitioners is for tartar emetic or arsenic to remove ascarids from the horse. Tartar emetic has the disadvantage of being a severe gastro-intestinal irritant and dangerous. Experimentally, I have found treatment with repeated doses of arsenic a slow and not very certain procedure.

As far as cattle are concerned, the only parasite I care to touch on at this time is stomach worm. This is the same worm that infests sheep, and just as the stomach worm does the greatest damage to lambs among sheep, so it does its greatest damage to calves among cattle. In districts where stomach worms are plentiful in sheep, it is practically certain that they will be plentiful in cattle, and under these circumstances calves should be treated for stomach worm. Experimental tests to determine the efficacy of treatments and the doses required are lacking as far as stomach worm in cattle is concerned. However, we know that the copper sulphate treatment is highly efficacious and safe against stomach worm in sheep, and we may assume that the same treatment would be efficacious and safe against stomach

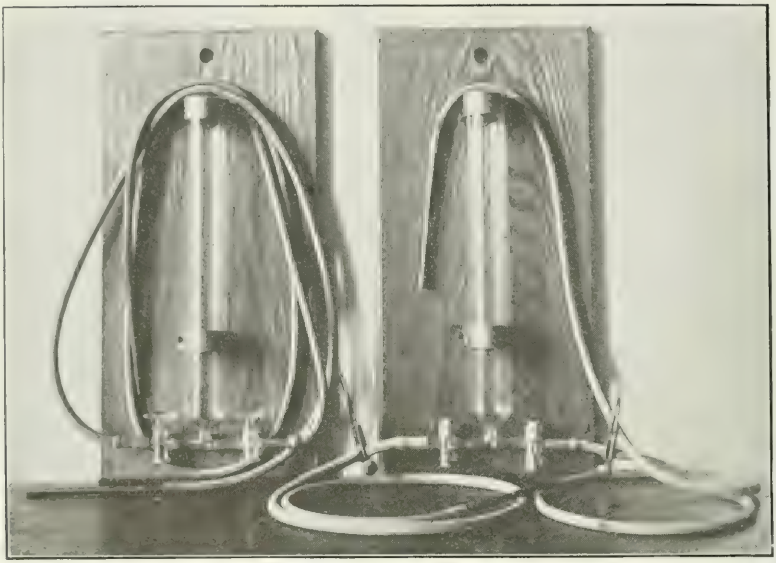

Fig. 1. Apparatus for drenching sheep for stomach worm. The apparatus at the right is the one with clip control on tubing.

worms in another ruminant in the appropriate dose, which dose can be computed reasonably well from the dose for a sheep. I prefer safe and conservative doses, repeated at long enough intervals to allow subsiding of inflammation and to avoid cumulative effects, to large doses. So I would start my dose for calves around 100 mils of 1 per cent solution in water for animals 2 to 3 months old, grading the dose up conservatively from this point, and repeating treatment at intervals of a month or 6 weeks from spring until after frost. The dose can be given with a metal dose syringe to calves under proper restraint, or if the number warrants it, more elaborate devices for administering the dose may be used.

Stomach worm in sheep is a well-known and serious pest. There are a number of treatments which have been recommended, among which may be mentioned the gasoline treatment, the creosote treatment and the copper sulphate treatment. In my opinion, the fact that gasoline is volatile and apt to enter the lungs and that it must be given three times in such comparatively expensive vehicles as milk, and in large amounts, precludes

its use so long as there is anything else that is free from these drawbacks. Most of the experiments on which I base my objection to gasoline and creosote have been published by Hall and Foster (1918). In the same paper will be found the experiments showing the advantages and efficacy of the copper sulphate treatment.

The copper sulphate treatment for stomach worms in sheep was devised by Hutcheon in South Africa and was very thoroughly tested. His reports cover the administration of the treatment to 23,000 sheep and show the good clinical results and the freedom from worms postmortem of sheep so treated. Our work in the Bureau of Animal Industry convinced us of the excellence of this treatment. Hall and Foster (1918) noted the use of 50-mil doses for lambs under 12 months old and 100 mils for those older, and described and figured an apparatus for administering the treatment. I am under the impression, based on our experiments and on some additional evidence obtained in Virginia and in Michigan in the control of stomach worms in sheep and goats, that stomach worms can be readily kept under control, at a point where it will have no discernible effect on the health of the sheep and perhaps even be eradicated from the range involved, by the administration of 50 mils of 1 per cent solution of copper sulphate every month or so except during the winter in localities where winter means freezing weather.

I have modified the apparatus originally described by Hall and Foster and now use this modification. This uses a shorter, thicker tube for the sake of compactness, is mounted on a board for the sake of convenience in hanging it up and in protecting the glass, has the inlet and outlet in glass as an integral part of the construction, partly for the sake of appearances and partly for the increased efficacy. Some earlier modifications use a glass control valve (Fig. 1), but this was too fragile, and the present apparatus, like the original, uses the clamp on the rubber tube. All apparatus is fed from a reservoir through one tube and delivers the dose to a metal tube, which is inserted in the sheep's mouth.

It is commonly stated by authorities that the copper sulphate crystals used in making this solution must be clear blue crystals, those having white patches or crusts on them to be rejected. In looking over my papers, I do not find the reason for this. I have

heard some reasons assigned, among others that the white patches were oxidized or insoluble, which is not the case, as the white patches represent copper sulphate which has lost part of its water of crystallization through efflorescence on exposure to air. Another reason which might be assigned, and this is perhaps the true reason, is that the loss of this water makes a difference in the amount of copper sulphate necessary in making up a solution, so that the weight of blue crystals, containing a rather large amount of actual $CuSO_4$ as the same weight of whitish material containing less water of crystallization. The fresh blue crystals of copper sulphate are $CuSO_4, 5H_2O$; on exposure to air the light-colored patches form and these have the formula $CuSO_4, 2H_2O$. At 100° C., $CuSO_4, H_2O$ is formed and at red heat $CuSO_4$. The differences in weight of these various forms of copper sulphate make considerable difference in the amount required to make a 1 per cent solution or any given strength. Thus to make a quart of the 1 per cent solution would require 0.946 gm. of the blue crystals, 0.747 gm. of the blue white powder formed on the crystals by efflorescence, or 0.6 gm. of the anhydrous copper sulphate formed at red heat. Hence in making a solution, it is important to know what strength is being made, and one should use only one sort of copper sulphate to insure this. The copper sulphate solution resulting, if of the required strength, will be the same, no matter what form of the salt is used.

Nodular worm infestation in sheep is a serious disease, which I mention only to express the opinion that we have not as yet established a really satisfactory treatment for it. In our experiments in the Bureau of Animal Industry, Hall and Foster found an efficacy of 17 per cent for chloroform and castor oil, 16 per cent for gasoline in milk, 9 per cent for chenopodium, 0.6 per cent for copper sulphate solution, and 0 per cent for powdered copper sulphate in capsule, and petroleum benzin in milk. The presence of the complex ruminant stomach and the fact that the adult nodular worm is in the cecum and colon remote from the mouth are facts that make oral medication for nodular worm a difficult matter. The bulk of the injury due to the worm is done in the larval stage, so that the removal of the adult worm, if accomplished, probably does little for the sheep, unless the anthelmintic treatment is part of an eradication program contemplating

adequate prophylaxis as well. Rectal medication might be used for the removal of the adult worms, but this is a slow procedure and less apt to be practical than oral medication.

Railliet (1915) notes the use of such a method by Brumpt. A preliminary dose of 25-30 gms. of sulphate of soda is given to the sheep to render the stools fluid. This being accomplished, the sheep is suspended by its hind legs and given a rectal injection of 1-1½ liters of water containing a thymol emulsion with the thymol at the rate of 1 gm. for each 3-5 kilos of weight of sheep; the anus is then held closed and the abdomen manipulated in such a way as to make the lavage penetrate and rinse out the intestine. This method is perhaps suitable for the patient workers of Europe with their sheep scattered in small flocks over many holdings, but it is not well adapted to the American temperament or the large flocks in this country.

The worm which is most generally recognized as a common drawback to swine-raising is the ascarid. These worms are extremely common in swine and are often present in large numbers. They are large worms and frequently may be seen in the intestines of swine at abattoirs in such numbers as to distend the small intestine, forming a sort of sausage with worms for the stuffing. In the experiment work carried on at Washington, chenopodium was found superior to any other drug for the removal of these worms. Oil of chenopodium may be used in about this dosage: Give pigs 1 mil of the oil for every 25 pounds of weight of pig up to 8 mils, following the dose immediately by a purgative, such as an ounce of castor oil for animals weighing up to 100 pounds, and double this amount for those weighing over 100 pounds. Be sure the animal is fasted a full 24 hours before treatment and not fed for two or three hours after treatment. The writer has seen abundant evidence of the necessity for observing this rule. Restraint for pigs is a more or less vexatious problem, but Foster found that he could dose 176 hogs in an 8-hour day, the animals ranging in size from young pigs to large boars and brood sows. We dosed pigs by pulling the jaws apart with two loops of heavy wire or rope. Another method which is used is to put one end of a short piece of old rubber garden hose in the pig's mouth and pour the dose through the hose as the pig chews on it.

While on this topic of dosing pigs, the writer would like to

again express the idea that mineral mixtures and stock tonics are inadequate and unsatisfactory substitutes for anthelmintic treatment. Experiments by Foster and myself in the Bureau of Animal Industry convinced us that mixtures of charcoal, lime, ashes, iron sulphate and such ingredients were of no value in removing worms or preventing worm infestation in swine, but the Bureau did not see fit to publish our conclusions. The reason which was given me for this was that these mineral mixtures were valuable in the bodily economy of the pig, and that if the farmer learned that they did not remove worms he might quit using them. This reasoning did not appeal to me at the time and does not appeal to me at this time. If a farmer wishes to feed mineral mixtures for the value of the mineral food content, he should do so, but he should not invest time or money in such mixtures for the purpose of clearing out or preventing worm infestations. If he does, he allows his pigs to remain wormy when they should be relieved from worms and puts money into something he may not need or want.

In the same category as the mineral mixture are most of the so-called stock tonics. Of these products, the Michigan Dairy and Food Department says:

"In recent years agricultural papers have been filled with advertisements of various stock tonics. * * * Wherever careful experimental trials have been made under expert and disinterested supervision * * * the outcome has invariably been that the use of condimental feeds as feeds was problematical and without material effect on production."

The products are about as ineffective in controlling worms. I fed one of the best known stock tonics to a 10-kilo dog, giving 11 doses in 16 days, using the dose for a 500-pound hog. In that period the dog passed 17 per cent of its ascarids and no *Dipylidium*. At the same rate it would have required 3 months to free the dog of its ascarids. Had it been given in food, as it was supposed to be, it would have been much less effective. Another well-known stock tonic was fed to a 10-kilo dog daily in the dose for a 100-pound hog. As this tonic is 95 per cent common table salt, the dog was unable to keep it down, so after 3 days' vomiting the dose was cut in half, and given daily for 14 days. No worms were passed, and the treatment was a failure. Another tonic for hogs was given to a 14.5-kilo dog, in the dose

for a 50- to 75-pound pig, giving 27 doses in 32 days, or double the number said to be necessary. This treatment was 6 per cent effective against ascarids and removed no *Tænia*. To be sure, a dog is not a hog, but the evidence as to anthelmintic ineffectiveness is none the less relevant.

Stock tonics must be safe for general use. Anthelmintics are not safe for general use, as a rule, if they are potent. Hence, stock tonics seldom contain the amounts of potent anthelmintics necessary to accomplish much.

The writer has been testing anthelmintics on dogs for three years and has made tests on 400 dogs. As a result of this work, certain anthelmintics for dogs have been rather firmly established as satisfactory.

The dog ascarids may be easily eliminated by the use of oil of chenopodium administered in a single dose of 0.1 mil per kilo of weight of dog. The oil may be given without enclosing it in a capsule, but this causes a lot of salivation, a very tenacious saliva when the chenopodium is accompanied by castor oil, which is the way it should be given. Hard capsules may be used for the oil, or the soft, elastic capsule. In my experimental work, I have found the soft capsule entirely satisfactory and prefer it to other forms of administering the drug. I use the dosage given by Hall (1917) when administering the soft capsules, namely, 5 minims to dogs weighing 10 pounds or less; 10 minims to dogs weighing 10 to 20 pounds; 15 minims to dogs weighing 20 to 30 pounds; not to exceed 20 minims to dogs weighing over 30 pounds; and for toy dogs, cut the dose to 2 or 3 minims. Give an ounce of castor oil to dogs other than toys, and give toys a half ounce; give the castor oil immediately after the chenopodium. This is important. Chenopodium is toxic, constipating, and a gastro-intestinal irritant. Castor oil slows absorption and distributes it over a larger surface of the gastro-intestinal mucosa, and it promotes elimination. Dogs can be given double the minimum lethal dose of oil of chenopodium with castor oil and will survive, as Hall (1918) has already noted. If dogs show symptoms of poisoning, which is not apt to be the case when castor oil is given with the chenopodium, give more castor oil. The following are contraindications for oil of chenopodium: Severe acute or chronic nephritis, organic heart trouble of certain

types, marked cachexia, severe gastro-enteritis, and severe infectious diseases, especially distemper. Nephritis is extremely common in dogs, as we know from such work as that of Meyer (1911) and as our post-mortem examinations constantly show. Normal kidneys are scarce, even in young dogs. Meyer notes that Siebel suggested that this condition was probably a sequel to distemper, that disease which seems always and everywhere present among dogs. The large amount of meat in a dog's diet may predispose to nephritis. Ordinarily, dogs with the customary chronic nephritis tolerate oil of chenopodium very well, but I have had one or two deaths among my dogs that I thought were due to the action of a therapeutic dose of chenopodium in causing an intensification of an already severe nephritis. Ziegler (1917) regards death from a lethal dose as due to acute nephritis; personally, I believe death is due to a combination of nephritis, gastro-enteritis and heart depression.

Organic heart trouble in dogs is rarely recognized or even looked for, and I have seen no cases where I could attribute the death of an animal to the presence of such a condition, but the fact that chenopodium acts terminally as a heart depressant indicates the danger in this quarter.

Cachexia in dogs is apt to be an accompaniment of parasitism, but it is a condition that calls for caution in the use of an anthelmintic. I have had a number of deaths occur from the use of therapeutic doses of chenopodium and other drugs in cachectic animals. Such animals should be put on a nourishing diet before treatment, but if it is necessary to administer anthelmintics immediately, use one which is not a gastro-intestinal irritant, if possible. For this purpose santonin is to be recommended. Santonin is not a drug which gives good results in single-dose treatments. Even when in large doses, and I have used such doses as a half-grain for every pound of weight of dog, santonin cannot be depended on to remove all the worms present. The correct way to use santonin, so far as tests indicate, is to give small doses daily for a number of days, then suspend treatment for a few days, and repeat if necessary. I find experimentally that such treatment can be depended on to remove ascarids without setting up gastro-intestinal irritation. So far I have found santonin a very safe drug when given with an equal amount of calomel, and I

have yet to see the first fatality from this combination. I gave one dog 61 grains of santonin and an equal amount of calomel in this way in 90 days; the animal lost some weight and lost hair around the eyes, neck, the axillæ and inguinal region and along the abdomen, but seemed in good health otherwise. Another dog was given 50½ grains in 18 days.

Gastro-enteritis is a contraindication for the use of oil of chenopodium for the reason that the oil is a gastro-intestinal irritant. It is a condition that occasionally complicates distemper.

Dogs suffering from distemper should not be given oil of chenopodium. The bacterial infection overburdens the kidneys and heart, frequently occasions gastro-enteritis, and leads in many cases to cachexia. Such animals do not tolerate anthelmintic treatment.

For the removal of whipworms from the dog, santonin is the best drug of which I am aware. It should be given, as noted by Hall (1917), in doses of a grain a day with an equal amount of calomel. I think it is safest to give it for a week, then suspend treatment for a week, then repeat as often as necessary. Some experiments along this line are given here:

Dog No. 110, a mongrel, weighing 13.6 kilos, was given a grain of santonin and an equal amount of calomel daily for a total of 6 grains in 8 days. The dog passed no worms and was found to have 2 whipworms on postmortem examination. The treatment was a failure, evidently due to not being persisted in.

Dog No. 111, a terrier, weighing 10 kilos, was given the same treatment for a total of 6 grains of santonin and of calomel in 8 days. The dog passed 29 ascarids the second day of the treatment, 2 the third, 1 the fourth, and 1 the seventh. On postmortem it had 1 ascarid and 1 whipworm. It will be noted that 1 ascarid did not come away until the seventh day and that another was still present post-mortem. Had the treatment been persisted in, it would have removed the other ascarid, probably in a day or two, and the whipworms in time.

Dog No. 108, a mongrel, weighing 9.5 kilos, was given santonin and calomel 1 grain each daily for a total of 12 grains of each in 15 days. The third day of treatment the dog passed the

posterior portion of a whipworm and the fourth day the anterior portion. On post-mortem the animal was free from worms.

Dog No. 71, a spaniel mongrel, weighing 12 kilos, was given 1 grain each of santonin and calomel for a total of 61 grains in 90 days. The ninth day of treatment the dog passed 1 whipworm. Post-mortem the dog had 32 hookworms and 4 *Dipylidium;* this confirms the dictum that santonin is of no value against hookworms and also indicates its lack of tæniacidal value, so far as *Dipylidium* is concerned. The dog lost a lot of hair, as already noted above, and had sores around its nose, but it was very active at all times.

Dog No. 120, a mongrel, weighing 13.5 kilos, was given 5 grains each of santonin and calomel daily for 5 days, and then the dose lessened, on account of the persistent vomiting, to 2½, 3 and 3½ grains daily for a total of 50½ grains in 18 days. On the fourth day of treatment the dog passed 14 whipworms. Post-mortem the dog was free from worms. This experiment and the preceding show the tolerance of the dog for santonin, when given with calomel, and also the need for persistent treatment in order to remove whipworms.

The most serious of the intestinal parasites of dogs is the hookworm. Hall and Foster (1918) found that chloroform at the rate of 0.2 mil per kilo, mixed with an ounce or so of castor oil, had an efficacy of 57 per cent against hookworm; oil of chenopodium at the rate of 0.1 to 0.3 mil per kilo, followed immediately by an ounce or so of castor oil, or given with castor oil, had an efficacy of 32 per cent; and thymol and calomel, in doses of 0.298 to 1.752 gm., had an efficacy of 15 per cent. In further tests of chloroform in our laboratory at Detroit, I have been unable to obtain as high efficacy as was obtained in the work at Washington. However, I have found that healthy dogs have a considerable tolerance for chloroform, surviving doses not only of 0.2, 0.3 and 0.4 mil per kilo, but also of 0.666 mil, 1.0 mil and 2.0 mils per kilo. I have been told by a physician that he has given chloroform in doses of a half-ounce to an ounce to patients. Alessandrini only uses 3-4 grams for man. The oral administration of chloroform produces an acute yellow necrosis of the liver, and this same condition is present and responsible for death in delayed chloroform poisoning from anesthesia. The

condition has been studied and described by Whipple and Sperry (1909). If the patient survives, the necrosis clears up in from 10 days to 3 weeks, leaving a practically normal liver. One of the Detroit dogs, No. 88, a collie mongrel, weighing 15 kilos, was given by stomach tube 30 mils of chloroform, a dose rate of 2 mils per kilo, in 40 mils of castor oil. Soon after dosing, the dog lay down, but was up and around in an hour and showed no symptoms. An hour and a half after dosing the dog vomited, and then lay down for a half-hour. After that the dog looked and acted entirely normal. Twenty-one days after this treatment the dog had a litter of 7 pups, and at least one of these pups was alive and well 4 months later. Fifty-five days after the chloroform was administered this dog was put in a chloroform box with 4 other dogs. The other dogs died inside of an hour. This dog survived the same atmosphere for almost 7 hours and then appeared to be coming out of the anesthesia; more chloroform was added and the dog presently succumbed.

Attempts to remove hookworms with single doses of oil of chenopodium did not meet with a high degree of success. The repeated administration of small doses, 2-5 minims daily for several days, gave good results, but apparently occasioned some little gastro-intestinal irritation. The method most used at present in the removal of hookworms from man, the administration of 3 doses at hour intervals, gave the best results. Some tests of this mode of treatment were as follows:

Dog No. 289, a hound, weighing 21 kilos, was given 3 doses, each dose consisting of a 10-minim soluble elastic capsule of oil of chenopodium, followed immediately by 15 mils of castor oil, at hour intervals, the last dose being followed an hour and a half later by 4 mils of chloroform in 15 mils of castor oil. The following day the dog passed 61 hookworms and 5 ascarids. The animal was killed the fourth day and found to have 10 hookworms. The treatment was therefore 86 per cent effective against hookworms and 100 per cent effective against ascarids.

Dog No. 301, a spaniel mongrel, weighing 15 kilos, was given the same treatment, except that no castor oil was given with each dose of chenopodium and the chloroform was given an hour after the last one in 30 mils of castor oil. The day after treatment the dog passed 33 hookworms and 1 ascarid. The third

day after treatment the dog was found dead. One hookworm was found post-mortem. The treatment was therefore 97 per cent effective against hookworms and 100 per cent effective against ascarids. This dog was in a late stage of distemper and anthelmintic treatment was contraindicated. Nursing would probably have saved the animal; the anthelmintic hastened death.

Dog No. 300, a wolfhound mongrel, weighing 18 kilos, was given 3 doses, each dose consisting of a 5-minim soluble elastic capsule of oil of chenopodium, at hour intervals, followed an hour later by 4 mils of chloroform in 30 mils of castor oil. The next day the dog passed 3 hookworms. The animal was killed the fourth day after treatment and found to have 3 hookworms, 1 *Physaloptera*, and 15 *Dipylidium*. The treatment was therefore 50 per cent effective against hookworms and 0 per cent effective against *Physaloptera* and *Dipylidium*.

Dog No. 292, a hound, weighing 14.5 kilos, was given the same treatment as Dog No. 300, except that each 5-minim capsule was accompanied by 15 mils of castor oil. The following day the dog passed 23 hookworms and the second day 7 hookworms. The animal was killed the fourth day and found to have 1 hookworm and 2 *Tænia pisiformis*. The treatment was therefore 97 per cent effective against hookworms and 0 per cent effective against *Tænia*.

Dog No. 293, a collie mongrel, weighing 12 kilos, was given 3 doses, each dose consisting of a 10-minim soluble elastic capsule of oil of chenopodium, at hour intervals, the last dose followed an hour later by 15 gm. Epsom salts in simple syrup. The next day the dog passed 2 hookworms and the second day 2 more hookworms. The animal was killed on the fourth day and found free from parasites. The treatment was therefore 100 per cent effective against hookworms.

Dog No. 294, a collie, weighing 19 kilos, was given the same amount of chenopodium in the same way, but the Epsom salts were omitted and one-third of a grain of cascarin was given with the first and third doses of chenopodium. Two days later the dog passed 2 hookworms and 1 whipworm and 4 days later passed 1 more hookworm. The animal was killed the fourth day and found to have 1 hookworm, 21 whipworms, and 6 *Tænia pisiformis*. The treatment was therefore 75 per cent effective against

hookworms, 5 per cent effective against whipworms, and 0 per cent effective against *Tænia*.

Dog No. 299, a mastiff mongrel, weighing 15 kilos, was given 3 doses of 10 minims of oil of chenopodium in soluble elastic capsules at hour intervals, each dose being followed by the feeding of uncooked meat, to ascertain the effect of the presence of food on the efficacy of the anthelmintic. The day following treatment the dog passed 3 hookworms and 5 ascarids. The dog was killed the fourth day and found to have 5 hookworms. The treatment was therefore 37.5 per cent effective against hookworms and 100 per cent effective against ascarids. The presence of food lessened the efficacy of the anthelmintic, as would be expected.

Dog No. 309, a foxhound, weighing 14 kilos, was given 3 doses of 10 minims of oil of chenopodium in soluble elastic capsules at half-hour intervals, followed a half-hour after the last dose by 30 mils of castor oil. After this treatment the dog broke out of its cage and got some meat. It passed no worms and was killed the fifth day after treatment. Post-mortem there were 22 hookworms and 6 whipworms. The treatment was therefore 0 per cent effective against hookworms and whipworms.

Summarizing the foregoing, it appears that very high efficacy against hookworms in the dog may be expected from the use of oil of chenopodium in 3 doses of 10 minims each for average-sized animals or larger ones, and of 5 minims each for smaller animals. Even the latter dose is too large for toys, and should be cut down according to the size and condition of the animal. The chenopodium seems to be quite effective whether given alone or with 15 mils of castor oil to each dose. I prefer to give the castor oil, as I believe it adds to the safety of the animal very materially. The hour interval seems to give more efficiency than the half-hour interval. Some purgative should be given not later than an hour after the last dose of chenopodium. I prefer the soluble elastic capsule to other forms of administration of the chenopodium for dogs. It is convenient and effective, so far as dozens of tests on dogs show. The addition of chloroform to a final dose of castor oil probably aids in removing additional worms.

Treatment for hookworm in dogs calls for considerable judg-

ment. Such animals already have an irritated intestine due to hookworm petechiæ, and, if clinical cases of uncinariasis or kennel anemia, are weak, emaciated and anemic. It is easy to kill such dogs by anthelmintic treatment. Hookworms are difficult to remove and call for larger doses of drugs than do ascarids. They will not respond to such drugs as santonin, which are non-irritant, and they require the use of such drugs as chenopodium, chloroform or thymol, all of which act more or less as gastro-intestinal irritants. Under these conditions, it may often be advisable to combine nursing treatment with repeated treatments by small doses of anthelmintic at intervals of two weeks or so, until the removal of part of the worms and the nursing put the animal in shape to endure the relatively drastic treatment necessary to clean out the infestation.

The worms which are of commonest occurrence in cats are the ascarids. These set up substantially the same chain of symptoms in cats as in dogs, except that the high-strung nervous system of the cat predisposes it to certain nervous disorders, and cats infested with worms are frequently subject to "fits." The treatment I have used and found successful for removing these worms is to give the cat a half-ounce of castor oil and then stick a pin in a 5-minim soluble elastic capsule of oil of chenopodium and squirt from 2 to 4 minims of the oil against the roof of the mouth, or against the tongue. This is safe and effective. One must use these small doses in treating cats in order to be on the safe side, as they are twice as susceptible to poisoning from chenopodium as are dogs, the minimum lethal dose per kilo being only half as large.

The common nematodes of poultry are the heterakids, including the large *Ascaridia* of the small intestine and the small *Heterakis* of the cecum. I have nothing to add here to the findings reported by Hall and Foster (1918), who found an efficacy against *Ascaridia* of 76 per cent for turpentine in 2-mil doses, mixed with an equal amount of olive oil and followed immediately by 8 mils of castor oil, and an efficacy of 69 per cent for oil of chenopodium in a dose of 0.2 mil mixed with 2 mils of castor oil and preceded by 2 mils of castor oil, and an efficacy against *Heterakis* of 19 per cent for chopped tobacco stems soaked in water and mixed with feed.

A consideration of the foregoing shows that in the present state of our knowledge our best anthelmintics for certain purposes are oil of chenopodium, which is perhaps the most valuable anthelmintic known; santonin, valuable where repeated doses are desired and gastro-intestinal irritation must be avoided; turpentine, which acts in some respects like a weaker oil of chenopodium; copper sulphate, valuable in the ruminants, where its emetic action is not manifested; and tobacco, which seems to be adapted to the peculiar task of removing heterakids from the ceca of poultry.

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(Reprint from *The Journal of Laboratory and Clinical Medicine*, Vol. IV, No. 7, April, 1919.)

ON THE DETECTION OF SMALL QUANTITIES OF TRICHLORO-TERTIARY-BUTYL ALCOHOL (CHLORETONE) IN THE FLUIDS AND TISSUES OF THE ANIMAL BODY.

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Chloretone is one of the few organic drugs which (after its administration to an animal) persists as such in the fluids and tissues of the body for any length of time after being absorbed, while its slight solubility in water, its volatility with steam, its comparative stability, and its property of forming needle-like crystals make it possible, under certain conditions, to recognize it readily.

In a previous article (*Journal of Biological Chemistry*, 1918, xxxiv, 263) I have given a method for detecting small quantities of chloretone, either alone or when associated with substances that interfere to a certain extent with its recognition, and which lends itself admirably to the detection of chloretone in the fluids and tissues of the body where few if any disturbing factors are present.

In the stomach or intestinal content, in the urine, or in certain tissues that grind up to a fine pulp, it is only necessary to pass steam through them, but in certain tissues, for example, the blood or serum, it is necessary to subject them to a preliminary digestion with pepsin hydrochloric acid before distilling with steam, since coagulation takes place, and the small amount of chloretone usually present may be prevented from passing over with the steam.

The chloretone may be administered through the stomach in the form of tablets, crystals or capsules, or intraperitoneally, dissolved in water, oil, alcohol, etc. Both methods of administration may be employed, but regardless of method em-

ployed, the animal passes under the influence of the drug very quickly, the rapidity of action being determined by the method of administration, the condition of the dog, etc. The saturated aqueous solution in sufficient quantity works quite rapidly when given through the stomach, the rapidity being influenced by the quantity of food in the stomach, as well as other factors.

In the experiments carried out the dose usually exceeded considerably the M.L.D. of 0.25 gm. per kilo. The smallest dose given was 0.13 gm., the largest 2 gm. per kilo of body weight. Since the dogs were to be killed eventually, an excessive dose could be given without danger, as the animals would live a number of hours even after the administration of a large excess of the drug.

Although chloretone has been recognized in the fluids and tissues of the body only when comparatively large doses have been given, it is to be presumed that it is present and could be detected when small doses were employed, but then only in those organs that have an apparent affinity for the drug.

Briefly, the general method of procedure consists in administering to the animal, according to weight, a sufficient amount of the drug to produce complete anesthesia, waiting usually from two and a half to three and a half hours (in some cases longer), bleeding from the carotid artery (saving the defibrinated blood), perfusing with saline in order to wash out the remaining blood so far as practicable, removing the organs, reducing them when necessary to a fine pulp, digesting if necessary with pepsin HCl, subjecting to steam distillation, boiling distillate (using a return flow condenser), and examining the lower third of the cooled portion of the condenser for the typical needle-shaped crystals of chloretone.

Although it may not be necessary always to subject the pulp to steam distillation, or to a preliminary digestion with pepsin hydrochloric acid, it is advisable to do this in order to detect the smaller amounts of the drug. When needle-shaped crystals are noted near the crest of the condensing steam, or in the cooled condenser, becoming evident sometimes only after removal of the flame, the presence of chloretone is positive.

The amount of chloretone present has been estimated by comparative tests and is only an approximation.

The following fluids and tissues have been examined: blood, brain, bile, heart, intestine and contents, kidneys, liver lungs, muscle, pancreas, spinal cord, spleen, stomach and contents, urine, and vagus nerves.

One fact stands out very prominently: the nervous tissue nearly without exception contains chloretone in varying amounts; while it is very frequently absent from the other tissues, and this would lead us to the supposition, which has been inferred but never proved relative to this hypnotic, that the nervous tissue has an affinity for it.

The greater part of the experimental work which follows was carried out a number of years ago when chloretone was first marketed as a hypnotic and anesthetic; but this work was not published at that time. Recently, owing to the wide use of the drug and the consequent new interest aroused in it, it was thought opportune to publish this older data, together with some recent experimental work.

Experimental

A. Recent Work

I. Weight of dog 13.5 kg. (Chloretone administered intraperitoneally.) Sixteen c.c. of a 40 per cent alcoholic solution, or 6.4 gm., given (0.47 gm. per kilo). Dosed 8:00 A. M. (11/8/17), going under in ½ hour. Bled at 8:30, 11/9/17, 80 c.c. of blood being obtained. Organs removed 10:15 A. M., nearly 26 hours after the administration of the drug.

The liquids (blood, stomach contents, and urine) were tested directly by placing in a suitable flask and passing steam through them. When about 100 c.c. of distillate had passed over, it was boiled over a free flame, using a return flow condenser. The solid tissues were ground very fine by either passing through a grinder or rubbing up with sand in a mortar and distilled with steam, etc.

It will be noted in this experiment that the dog died 26 hours after the administration of the drug. Presumably most of it had been destroyed.

(1) Brain (wt. 46 gm.)	Positive (slight trace)
(2) Blood (80 c.c. defibrinated)	Negative
(3) Fibrin	"
(4) Liver	"
(5) Lungs	"
(6) Spleen	"
(7) Stomach contents	"
(8) Urine	Very slight trace (?)

II. Weight of dog 8 kg. Chloretone given per stomach; 3.2 gm. of chloretone given in capsules at 1:45 P.M.; at 4 P.M. 2 gm. given in 40 per cent alcohol, intraperitoneally. (0.65 gm. per kilo.)

At 4:10 P.M. dog bled from carotid artery (blood defibrinated). Organs removed two and a half hours after administration of drug.

Method of procedure same as in I.

(1) Brain (wt. 72 gm.)	Positive (½ mg.)
(2) Blood (defibrinated) (300 c.c.)	Negative
(3) Kidneys	Positive (¼ mg.)
(4) Liver	Negative
(5) Lungs	"
(6) Spleen (enlarged)	"
(7) Stomach contents	Positive (30 mg.)
(8) Urine	Negative

III. Weight of dog 7.5 kg. Chloretone given per stomach; 7.5 gm. given in capsules at 10:10 A.M. (1 gm. per kilo.)

Dog was completely under one hour later, and 3½ hours later was bled from the carotid artery, 250 c.c. of blood being taken. Warm saline solution was introduced into the femoral vein until 1200 c.c. had passed through and the wash collected from the carotid artery.

Organs removed 3½ hours after administration of the drug.

The stomach contents and the urine were distilled directly; the remaining organs were ground up and digested with pepsin HCl (250 c.c.) for two days. *Badly decomposed.*

The absence of any chloretone in all of the digested products is attributed to the fact that the flasks being insecurely corked allowed the chloretone to escape, and putrefaction to take place.

(1) Brain	74 gm.	Negative
(2) Blood	150 c.c.	"
(3) Heart	41 gm.	"
(4) Kidneys	21 gm.	"
(5) Liver	240 gm.	"
(6) Lungs	67 gm.	"
(7) Spleen	18 gm.	"
(8) Stomach contents	50 c.c.	Positive
(9) Urine	60 c.c.	Negative

IV. Weight of dog 11.5 kg. Chloretone given per stomach; 3 gm. of chloretone in aqueous solution and suspension given at 10:20 A.M.

At 10:30 A.M. 5 gm. of chloretone in capsules given (0.7 gm. per kilo).

Dog gradually became completely anesthetized, and 3 hours after the last dosing was bled and perfused with about two liters of saline solution. Dog died and autopsy showed that pressure of the perfusing fluid had been so great that it had caused a severe pulmonary hemorrhage.

Organs removed 3 hours after administration of drug. The urine and stomach contents distilled at once; the organs digested.

(1) Brain	Positive (50-100 mg.)
(2) Blood	Negative
(3) Heart	Very slight trace
(4) Kidneys	Positive (very slight trace)
(5) Liver	Negative (accident)
(6) Lungs	Positive (very slight trace)
(7) Spinal cord	Positive (5-10 mg.)
(8) Spleen	Very slight trace
(9) Stomach contents	Positive (300 mg.)
(10) Urine	Negative

V. Weight of dog 9½ kg. Chloretone administered per stomach; 5 gm. given in aqueous alcoholic suspension (250 c.c.) by means of a stomach tube. (0.35 gm. per kilo.)

Was bled from the carotid artery, just three hours after being dosed. The brain, spinal cord, and the vagi nerves were removed three hours after administration of the drug and ground up with sand and each digested with 250 c.c. of pepsin HCl for 16 hours in incubator at 37° C. Worked up in the usual way.

(1) Brain	Positive	20 mg.
(2) Nerves (vagi)	"	trace
(3) Spinal cord	"	5 mg.

B. Older Work

VI. Weight of dog 7 kg. Chloretone given per stomach; 300 c.c. of aqueous chloretone solution 0.8 per cent or 2.4 gm. given (0.33 gm. per kilo). Went under in 1 hour. At the end of 2 hours, dog bled from the carotid artery (portion of blood saved) and an unsuccessful attempt made to wash out the residual blood from the body with saline solution.

Contents of stomach and intestine (upper part) were tested directly.

The organs were removed, washed to free them as much as practicable from blood, and then ground up and digested several days with pepsin hydrochloric acid, 500 c.c. of 0.4 per cent HCl and 2 gm. of pepsin (1:3000) being used in each case.

(1) Brain	30 mg. estimated
(2) Blood	40 " "
(3) Heart	5 " "
(4) Kidneys	Slight trace
(5) Liver	" "
(6) Lungs	None
(7) Muscle	Slight trace
(8) Spleen	" "
(9) Stomach contents	500 mg.
(10) Intestinal contents	400 mg.

VII. Weight of rabbit ¾ kilo. Chloretone given per stomach; 1.5 gm of chloretone given (2 gm. per kilo). Animal died soon after.

Brain removed and freed of blood as much as possible by washing in water.

Digested in pepsin HCl solution for 5 days. Treated in the usual way with steam, etc.

Brain	10 mg.

VIII. Weight of dog 6 kg. Administered per stomach; 100 c.c. of 0.8 per cent chloretone solution given, or 800 mg. (0.13 gm. per kilo). After 2 hours cannula was inserted into carotid artery and blood washed out as far as feasible from head and brain and the latter placed in refrigerator over night.

One-half of the brain was reduced to a pulp; distilled with steam, etc.

The other half was reduced to a pulp and digested with pepsin HCl (1 gm. pepsin 1:6000) for 4 days.

(1) Brain (direct)	Negative
(2) " digested	40-60 mg.

IX. Weight of dog 11 kg. Chloretone given per stomach; 150 c.c. of 0.8 per cent chloretone solution given: 1½ hours later 250 c.c. more given; finally ½ hour later 250 c.c., 6 gm. in all (0.55 per kilo).

(1) Brain	Crystals of chloretone 10 mg.
(2) Blood	Negative
(3) Heart	"
(4) Kidneys	"
(5) Lungs	"

X. Weight of dog 22½ kg. Given per stomach. Twenty 3-grain chloretone tablets and 500 c.c. of a 0.8 per cent solution of chloretone, 8 gm. in all (0.35 gm. per kilo).

In half an hour dog was completely under. Bled from carotid artery (200 c.c. blood obtained). Stomach and bladder removed.

(1) Blood	Positive
(2) Stomach contents	"
(3) Urine	Negative

XI. Weight of dog 6.4 kg. Chloretone administered per stomach; 320 c.c. of 0.8 per cent chloretone solution or 2.56 gm. total (0.4 gm. per kilo). Killed after 2 hours.

Removed organs after washing out with saline solution.

(1) Brain	10 mg.
(2) Blood	3-4 mg.
(3) Heart	2 mg.
(4) Intestinal wall	Negative
(5) Intestinal contents	0.5 mg.
(6) Kidneys	5 mg.
(7) Liver	10 mg.
(8) Lungs	1 mg.
(9) Pancreas	Negative
(10) Spleen	Negative
(11) Stomach	5 mg.
(12) Stomach contents	150 mg.
(13) Urine	Negative

XII. Weight of dog 9 kg. Chloretone administered per stomach; 350 c.c. of 0.8 per cent chloretone solution or 2.6 gm. (0.29 gm. per kilo) given. Killed after 1½ hours.

Brain removed and worked up in the usual manner. One-half distilled direct with steam; the other half distilled after digestion with pepsin HCl.

Blood and urine were also tested.

(1) Brain	50 mg. (100 mg. total)
(2) Brain (digested)	2.3 mg.
(3) Blood (digested)	5 mg.
(4) Urine tested for acetone	Negative

XIII. Weight of dog 9 kg. Administered per stomach. Had been used for digitalis work. Two hundred fifty c.c. of chloretone solution 0.8 per cent given, or 2 gm. (0.22 gm. per kilo). Killed after 3½ hours.

Brain divided into two portions and treated in the usual manner.

Heart and muscle also tested after digestion.

(1) Brain (digested)	2-3 mg. (4-6 mg. total)
(2) Brain (undigested)	Negative
(3) Heart (digested)	"
(4) Muscle (digested)	1 mg.

XIV. Wt. of dog 9 kg. Administered per stomach. Had been used for digitalis work.

Three hundred seventy-five c.c. of chloretone solution 0.8 per cent given; 250 c.c. given at first, did not go under; later 125 c.c. given (0.33 gm. per kilo). Went under then in a few minutes. In about 5 hours brain removed, ground fine, and divided into two portions—one portion was digested in the usual manner; the other portion was treated at once.

(1) Brain (digested)	Positive 5 mg. (10 mg. total)
(2) Brain (undigested)	Negative

XV. Gave dog chloretone in aqueous solution 0.8 per cent 0.2 gm. per kilo of body weight (per stomach). Killed two hours after administration by bleeding. Blood washed out of circulatory system by saline solution before removal of organs. Organs washed also after removal. Blood was saved and tested for chloretone.

Organs ground fine and digested for 48 hours in 500 c.c. of water + 5 c.c. HCl (con.) and 2 gm. of pepsin U. S. P. at a temperature of 38° to 40° C. Each digestive mixture was distilled with steam, etc., in the usual way.

	Weight Gm.	Chloretone
(1) Brain	59	20-30 mg.
(2) Blood		Negative
(3) Bile		Mere trace
(4) Heart	85	10 mg.
(5) Intestinal walls		100 mg.
(6) Intestinal contents		30 mg.
(7) Kidneys	50	10 mg.
(8) Liver	234	Negative
(9) Lungs	120	100 mg.
(10) Pancreas		Negative
(11) Spleen	37	5 mg.
(12) Stomach		20 mg.
(13) Stomach contents	1900 c.c.	250 mg.
(14) Urine		Negative

XVI. Gave dog same amount as in XV, all conditions same.

(1) Brain	20-30 mg.
(2) Blood	Negative
(3) Bile (did not save)	
(4) Heart	10 mg.
(5) Intestinal wall	100 mg.
(6) Intestinal contents	10 mg.
(7) Kidneys	10 mg.
(8) Liver	Slight trace
(9) Lungs	Negative
(10) Pancreas	"
(11) Spleen	5 mg.
(12) Stomach	2 mg.
(13) Stomach contents	300 mg.
(14) Urine	Negative

XVII. Weight of dog 8.2 kg. Administered per stomach; continual dosage. Four tablets daily (3 grains), 2 in morning and 2 in afternoon, or total of 60 grains = 4 gm. (0.50 gm. per kilo).

Date		Weight	Pulse	Temp.	Dose	Remarks
6/29	A.M.	8200 gm.	96	103.2	6 gr.	
	P.M.	8640 "	132	101.4	"	
6/30	A.M.	" "	144	101.4	"	
	P.M.	" "	156	101.0	"	
7/1	A.M.	9090 "	168	101.2	"	
	P.M.		168		"	
7/3	A.M.	8640 "	120	99.8	"	Weak, does not eat.
	P.M.	7730 "	168	104.4	"	
7/5	A.M.	6000 "	136	100.0	"	Does not stand.
	P.M.	6400 "	...	101.4	"	
7/6	A.M.		...	.....		Found dead.

POST-MORTEM.

Dog had been dead overnight and it was consequently difficult to distinguish anything abnormal during life from post-mortem signs. Bladder greatly distended, containing 240 c.c. of urine. Acid in reaction. Sp. g. 1.030. Cloudiness, probably mucus or phosphates. The organs were ground up and treated in the usual way after digesting with pepsin HCl.

(1) Brain	1 mg.
(2) Blood	Negative
(3) Heart	Very slight trace
(4) Kidneys	Trace
(5) Liver	Spoiled, not worked up
(6) Lungs	" " " "
(7) Spinal cord	Negative
(8) Spleen	"
(9) Stomach contents	1 mg.
(10) Urine	Negative

XVIII. Weight of dog 5.5 kilo. Chloretone given per stomach; 300 c.c. chloretone solution 0.8 per cent. Anesthetized in 5 minutes. Was killed at the end of two hours by bleeding. Blood saved and organs removed from the body and freed from blood, so far as possible, by washing first with saline and then with hydrant water. Digested with pepsin HCl for a few days.

(1) Brain	20-30 mg.
(2) Blood	20 mg.
(3) Heart	1 mg.
(4) Intestinal contents	Negative
(5) Kidneys	3-4 mg.
(6) Liver	Negative
(7) Lungs	1 mg.
(8) Spinal cord	?
(9) Spleen	?
(10) Stomach contents	30 mg.
(11) Urine	Negative

XIX. Weight of dog 11 kilo. Chloretone given per stomach; 600 c.c. of chloretone solution 0.8 per cent given, or 4.8 gm. (0.44 gm. per kilo). Anesthetized in 5 minutes. Remained under all day, sleeping quietly. Killed 6½ hours after drug was given. Bled from carotid artery and vascular system washed out with several gallons of normal saline solution. Dog was still breathing while perfusion was carried on. The organs were thus quite thoroughly washed free from blood. The following organs were re-

moved, further washed with saline solution and tested for chloretone. Each organ was cut into small pieces and digested with pepsin HCl.

It was thought that washing out the organs removed the chloretone, still there was no chloretone found in blood before washing, or the drug may have been eliminated in 6½ hours. *The organs were incompletely digested.*

(1) Brain	Negative
(2) Blood	Negative
(3) Heart	Negative
(4) Kidneys	Negative
(5) Liver	Negative
(6) Lungs	Slight trace (?)
(7) Spinal cord	Negative
(8) Spleen	Negative
(9) Stomach contents	Negative
(10) Urine	Negative

The presence or absence of chloretone in the summary table is indicated by the plus (+) or minus (—) sign. Where the space is blank the organ was not tested; and where a positive sign is followed by a number the latter indicates the estimated amount of chloretone in mg., while the letter (t) or a question mark (?) indicates a trace or a questionable trace. In the fifth column the letter *p* following "bled" shows that the animal was perfused; while in the fourth column "time elapsed" is the time intervening between the commencement of administration and the death of the animal. In II the drug was administered per stomach and intraperitoneally; in I intraperitoneally only; in all others per stomach.

An examination of the table shows that chloretone is uniformly present under varying conditions in the brain and in larger amounts and more constantly than in any other organ of the body, where the organ is taken in its entirety, there being all the way from a trace to 100 mg. or an average of about 24 to 25 mg.

When this measure is applied to the other organs and fluids we find the brain contents is only exceeded by the intestine and intestinal and stomach contents. Here we would naturally expect to find an abundance of the drug (when given per stomach), depending on the amount administered, the length of time before the animal is killed, etc. Leaving these latter out of consideration, we find that the brain contents several times as much as the other organs.

DOGS	WT. IN KILO	DOSE PER KILO	TIME ELAPSED HOURS	MANNER OF DEATH	DIGESTED WITH PEPSIN	BRAIN MG.	BLOOD DEFIBRINATED MG.	FIBRIN MG.	GALL MG.	HEART MG.	INTESTINE (UPPER PART) MG.	INTESTINAL CONTENTS MG.
I	13.5	0.48	26.0	Died	No	+t	—	—				
II	8	0.65	2.5	Bled	"	+0.5				—		
III	7.5	1.00	3.5	"	Yes	—	—			+t		
IV	11.5	0.70	3.0	" p	"	+100	—					
V	9.5	0.53	3.0	Bled	"	+20	—			+5		+400
VI	7.0	0.33	2.0	"	"	+30	+40					
VII	0.75	2.00	1.0		"	+10						
VIII	6.0	0.13	2.0	Bled	"	+60				—		
IX	11.0	0.54	2.0			+10	—					
X	22.5	0.35	0.5	"			+			+2	—	+0.5
XI	6.4	0.40	2.0	" p		+10	+4					
XII	9.0	0.31	1.5		Yes	+100	+5			—		
XIII	9.0	0.22	3.5		"	+3			—			
XIV	9.0	0.50	5.0		"	+10			+t	+10	+100	+30
XV		0.20	2.0	Bled p	"	+30	—			+10	+100	+10
XVI		0.20	2.0	" "	"	+30	—			+t		
XVII	8.2	0.50	84.0		"	+1	—			+1		
XVIII	5.5	0.43	2.0	Bled p	"	+30	+20			—		
XIX	11.0	0.44	6.5	" "	"		—					
Average number of mg. found						24-25	4.9	t	0	2.5	66	90.1

Perfusion does not apparently lower the chloretone content of the organs.

In most of the experiments the organs were digested with pepsin hydrochloric acid, and this procedure *usually* increased the amount of chloretone obtained. The only exceptions are found in III and XIX, where decomposition had taken place during digestion in the former, and where incomplete digestion was found in the latter. In one instance (XII), the half of the brain digested contained less chloretone than the half undigested.

It will be noted that in I only a trace of the drug was found in the brain and none in the other organs. Since the organs were removed 26 hours after dosing, it is to be presumed that the chloretone had been destroyed or eliminated.

In II a portion of the drug was given intraperitoneally dissolved in alcohol, and its presence in only a small amount (0.5 mg.) in the brain after 2½ hours is explained by presuming that insufficient time for its absorption had been allowed.

In XVII 84 hours had elapsed, which would account for

TABLE

KIDNEY MG.	LIVER MG.	LUNGS MG.	MUSCLE MG.	PANCREAS MG.	SPLEEN MG.	STOMACH MG.	STOMACH CONTENTS MG.	SPINAL CORD MG.	URINE MG.	V. NERVE MG.	REMARKS
	—	—			—				+?		
+t	—	—			—		+50		—		
—	—	—			—		+t		—		Flasks not
+t	—	+t			+t		+300	+10	—		corked tightly.
								+5		+t	(decomposition)
+t	+	—	+t		+t		+500				
		—									
							+		—		
+5	+10	+1		—	—	+5	+150		—		
									—		
			+1								
+10	—	+100		—	+5	+20	+250		—		
+10	+t	—		—	+5	+2	+300		—		Fed for a
+t					—		+1	—	—		number of
+4	—	+1			—?	+	+30	+?	—		days.
—	—	—?			—	—	—	—	—		
2.6	1	9.2	0.5	0	0.9	5.4	130	3	0?	t	

the presence of only 1 mg. in the brain; while when we come to XIX, we find that the organs were very incompletely digested and that a rather long period of time ($6\frac{1}{2}$ hours) had elapsed.

In the spinal cord the drug was uniformly present except in XVII and XIX, where the reason for its absence can be explained. In the vagi nerves the drug was found positively in V. the only one tested.

It must be kept in mind also that idiosyncrasy plays a part in relation to this, as well as all other drugs, and may explain some of the variations in amount obtained in the tissues.

The experimental data above emphasizes the fact that the nervous tissue, especially the brain, seems to have a selective action for chloretone, which would explain the hypnotic and anesthetic properties of the drug in a large measure. So far as I am familiar with the literature, this is one of the comparatively few instances in which the presence of drugs has been positively proven in the nervous tissues, although this has generally been assumed for a long time, and it is

only possible in this case on account of the specific properties of chloretone.

The experimental data raises the question also of the selective action of hypnotics and anesthetics as well as other drugs, but places beyond dispute the selective action of this drug for the cells of nervous tissue, and makes probable the assumption of the selective action of other drugs for the tissues.

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 201, 1919.

(Reprinted from the *Cornell Veterinarian*, Vol. 9, No. 2, April, 1919.)

A NOTE IN REGARD TO THE SEASONAL APPEARANCE OF ANOPLOCEPHALA MAMMILANA.

H. Preston Hoskins, V.M.D.

(From the Medical Research Laboratories, Parke, Davis & Co., Detroit, Mich.)

With Hall[1] the present writer recently reported finding the rare horse tapeworm Anoplocephala mammilana in two subjects at Rochester, Mich. Since that report was prepared, this parasite has been found in two more horses at the same place, making four cases in all.

The following table gives the data concerning these:

Horse No.	Date Autopsy	Number of Specimens	Months at Parkedale Farm
1019	4-19-18	37	11
686	5-2-18	1	21
988	5-21-18	5	11
275	6-4-18	3	40

In the course of some investigations into the causes of deaths of horses used in the production of various antitoxins and antibacterial sera, the author has had an opportunity to hold autopsies on a considerable number of horses during the past eighteen months. In addition to these autopsies, a number have been held on horses destroyed for various purposes, or bled to death because they were no longer yielding a potent serum. When such horses showed no abnormalities at autopsy, the serum was used for the preparation of culture media. In the great majority of these autopsies an examination of the gastro-intestinal canal was a part of the routine procedure. In a few cases it was omitted for certain reasons. The autopsy notes usually state the presence of any parasitic infestation, although bots, ascarids and strongyles were so com-

[1]Hall, M. C., and Hoskins, H. Preston, 1918. The Occurrence of Tapeworms, Anoplocephala Spp., of the Horse in the United States. Cornell Veterinarian, VIII (4), October, pp. 287-292.

monly met as to receive little more than passing notice. It is not considered likely that any great number of tapeworms failed to be observed.

In connection with the finding of the tapeworms, however, an interesting point is brought out, namely, the seasonal appearance of this parasite, and in view of the fact that the life history of Anoplocephala spp. is not known, the writer has thought it worth while to report on this point. The four horses which revealed the presence of the parasite were all autopsied between April 19th and June 4th. (Several horses were autopsied during this period which were free from the parasite.) Previous to April 19th and subsequent to June 4th the parasite has not been encountered, although upwards of one hundred horses have been examined in the five months since June 4th, and approximately a year prior to April 19th, all on the same premises, receiving the same food, water and general attention.

The horses had been pastured during the previous summer. Horse lice were usually prevalent during the winter, but our records do not show whether any of the four horses in which tapeworms were found were among those infested with lice. Perhaps this is unimportant, but it has been thought worth while to call attention to all facts which might have any possible value for future investigations. Meanwhile a close watch for tapeworms is being kept, and it will be very interesting to observe whether specimens will make their appearance during 1919, at about the same time as they did during 1918.

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 202, 1919

(Reprinted from *Rhodora, Journal of the New England Botanical Club*, Vol. 21, 1919.)

BROMELICA (THURBER): A NEW GENUS OF GRASSES.

Oliver Atkins Farwell.

(Department of Botany, Parke, Davis & Co., Detroit, Mich.)

For some years past our eastern species of Oat Grass have been bandied about between *Avena* and *Melica*, affording for some a merry game of shuttlecock. These species appear to have no permanent home and to be a restless group, that, like Banquo's Ghost, will not down. It seems best, therefore, to create a new genus for them. At least one of that small group of grasses, to which belong our eastern Oat Grasses, has been included at one time or another in five different genera, *Festuca, Bromus, Melica, Avena* and *Trisetum*. As regards our eastern species Michaux first described *Avena striata* in 1803; Torrey next described it as *Trisetum purpurascens;* A. Gray replaced it in *Avena*, using Michaux's name; Hitchcock then removed it to *Melica* as *M. striata;* finally Nash restored it to *Avena* as *A. Torreyi*. The second species was described by Porter in 1867 as *Avena Smithii* and it was removed to *Melica* by Vasey in 1888. At the present time Hitchcock, in Gray's Manual, lists these species under *Melica;* Britton & Brown in the Illustrated Flora list them under *Avena;* Rydberg in the Flora of the Rocky Mountains steers an intermediate course, listing the first under *Avena* and the second under *Melica*. When authors are at such wide variance with each other in their treatment of such closely related species, the probabilities are that the species do not belong to any one of the genera to which they have been referred. A careful analysis of the distinguishing characters of each genus bears out this supposition.

These species can scarcely belong to *Avena* since they lack the most important *tribal characters* distinctive of the *Aveneæ*, viz.: the spine-like end of the rachilla prolonged behind the uppermost floret and glumes *longer* than the lower floret. They do agree with the *Festuceæ* in not possessing the spine-like elongation of the rachilla and in having glumes shorter than the lower floret. A

genus of the *Festuceae* must then be sought for these species and amongst those genera having many nerved lemmas. They do not belong to *Festuca* because the lemmas are *not entire.* They do not belong to *Bromus* because the grain is *not adherent to the palet nor pubescent* at the summit. They do not belong to *Melica* because the *lemmas are not subcoriaceous* and the uppermost *do not form a convolute club-shaped mass* but *are distinct.* In *Bromelica* the glumes and lemmas are *membranous,* the former being somewhat *unequal and shorter* than the lowest floret; the latter are *acute, notched or bidentate,* generally with a *terminal awn* formed by the excurrent midrib between the teeth, the uppermost being *similar to the others and distinct,* the uppermost floret consisting of a single lemma only. Thus delimited, *Melica* and *Bromelica* consist, each of a clear, homogeneous group of species; united, *Melica* is a heterogeneous group. *Bromelica* is almost exactly intermediate between *Melica* and *Bromus,* with closer relationship to the latter than to the former, which is exemplified by habit and by the characters of the glumes and lemmas; if *Bromelica* is retained in *Melica* there is no good reason why *Melica* in its entirety should not be united with *Bromus.*

Lemmas membranous, all alike and distinct, acute, awned or awnless.
  Lemmas entire .... *Festuca.*
  Lemmas notched or bidentate.
    Grain adherent to the palet and pubescent at apex .... *Bromus.*
    Grain free, not pubescent .... *Bromelica.*
Lemmas subcoriaceous, obtuse, convolute around each other and forming a club-shaped mass .... *Melica.*

The synonymy and species follow:

BROMELICA (Thurber), n. gen. *Melica* subgenus *Bromelica* Thurber, Bot. Calif. ii, 304 (1880), and in Gray's Manual, ed. 6, 152 (1908).

B. striata (Mx.), n. comb. *Avena striata* Mx. Fl. Bor. Am. i, 73 (1803). *Trisetum purpurascens* Torr. Fl. U. S. 127 (1824). *Melica striata* (Mx.) Hitch. Rhodora, viii, 211 (1906). *Avena Torreyi Nash* in Britt. & Br. Illus. Fl. 2, i, 219 (1913).

B. Smithii (Porter), n. comb. *Avena Smithii* Porter in Gray's Manual, ed. 5, 640 (1867). *Melica Smithii* (Porter) Vasey, Bull. Torr. Cl. xv, 294 (1888).

B. aristata (Thurber), n. comb. *Melica aristata* Thurber in Boland. Proc. Cal. Acad. iv, 103 (1870).

B. subulata (Bong.), n. comb. *Festuca subulata* Bong. Veg. Sitch. 173 (1832). *Bromus subulatus* Griseb. in Ledeb. Fl. Ross. iv, 358 (1853). *Melica acuminata* Boland. Proc. Cal. Acad. iv, 104 (1870). *M. subulata* Scribn. Proc. Acad. Phila. 47 (1885).

B. Harfordii (Boland), n. comb. *Melica Harfordii* Boland. Proc. Cal. Acad. 47 (1885).

B. Harfordii, var. **minor** (Vasey), n. comb. *Melica Harfordii,* var. *minor* Vasey, Bull. Torr. Cl. xv, 48 (1888). *M. Harfordii,* subsp. *tenuior* Piper, Cont. U. S. Nat. Herb. xi, 127 (1906).

B. Geyeri (Munro), n. comb. *Melica Geyeri* Munro in Boland. Proc. Cal. Acad. iv, 103 (1870). *M. bromoides* Boland. ex. A. Gray, Proc. Am. Acad. viii, 409 (1872).

B. Geyeri, var. **Howellii** (Scribn.), n. comb. *Melica bromoides,* var. *Howellii* Scribn. Proc. Acad. Philad. 47 (1885).

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 203, 1919.

(Reprinted from the *Journal of Biological Chemistry*, Vol. XXXVIII, No. 1, 1919.)

IS LACTALBUMIN A COMPLETE PROTEIN FOR GROWTH?

By A. D. Emmett and G. O. Luros.

Recent reports of investigations suggest that there is a difference of opinion as to the growth-promoting value of lactalbumin. We are especially interested (1) in this point since many of our findings as to the relative value of certain vitamin preparations, when added to an otherwise complete diet, have been based upon the assumption that our basal protein, lactalbumin, is capable of promoting growth; that is, that it contains the fundamentals for furnishing *all* the protein complexes required.

Osborne and Mendel (2) have shown very clearly that they were able under their conditions to obtain excellent results with lactalbumin as the primary protein in their diets. In fact, they (3) found in comparing the food value of edestin, casein, and lactalbumin for the growth of young rats that "the superior efficiency of the lactalbumin in the nutrition of growth is demonstrated beyond question." "Thus, to produce the same gain in body weight, 50 per cent more casein than lactalbumin was required, and of edestin nearly 90 per cent more." In a recent article, Osborne, Mendel, and Ferry (4) suggested a method of calculating the growth-promoting value of various proteins in rations. This method was based upon correlating the gain in body weight with the grams of both total food and protein consumed. When the authors compared their results for casein and lactalbumin on this basis, they found that the same gains for these two proteins could be made, over the same length of time, only when the casein-fed rats consumed 20 and 24 per cent more of ration and protein respectively than did the lactalbumin-fed rats.

On the other hand, McCollum, Simmonds, and Parsons (5) have taken the stand that lactalbumin is an incomplete and

poor protein for growing rats. Their conclusion was based upon the fact that when they compared casein with lactalbumin in two rations which were alike in all other respects, normal growth resulted in the first instance and almost no growth in the latter instance. Their basal diet was made up of peas 15 per cent, salt mixture 1.8 per cent, agar-agar 1 per cent, dextrin 38 per cent, and butter fat 5.0 per cent; 9 per cent of lactalbumin and of casein was added, respectively, to two portions of this basal ration, bringing the total protein up to about 18 per cent. The Osborne and Mendel rations consisted of 18 per cent protein (lactalbumin or casein), 28 per cent protein-free milk, 28 per cent starch, 18 per cent butter fat, and 10 per cent lard. McCollum and his associates stated:

> "We are forced to the conclusion that lactalbumin is a poorly constituted or an incomplete protein and that the excellent results of Osborne and Mendel were due to the high proportion of nitrogen derived from the 'protein-free milk' which was present in their food mixtures and served to supplement the lactalbumin with respect to some as yet undetermined cleavage product which is essential for growth."

According to Osborne and Mendel, their protein-free milk was made up of 80 per cent lactose, 15 per cent inorganic salts, 2.2 per cent protein, 2.7 per cent non-protein matter, and carried the water-soluble B vitamin. Mitchell and Nelson (6) found that they could reduce the per cent of protein nitrogen in the "protein-free milk" by neutralizing the boiling milk whey; but by this procedure they contaminated the lactalbumin, since calcium phosphates were thrown down at the same time. Kennedy (7) had shown that the Osborne and Mendel protein-free milk contains "either unprecipitated protein or peptids of considerable size;" that about half of the nitrogen was precipitated by certain salts in the non-amino form, and that after tryptic digestion there was an increase in amino nitrogen.

From the above facts, it is evident that protein-free milk is a complex mixture and possibly capable of carrying the proper kind of protein or nitrogenous cleavage products to furnish the nitrogen supplement that may be needed for an incomplete protein. Whether there would be a sufficient *amount* of this nitrogenous supplement present is another question.

DISCUSSION.

In using lactalbumin as the sole protein in the diet of young rats and as a supplement to an incomplete protein for growth, we have obtained results which agree with Osborne and Mendel (Charts 1, 2, and 3). On the other hand, we have recorded data with lactalbumin which correspond to what McCollum, Simmonds, and Parsons found (Chart 4). That is, we have secured in many cases normal growth, and then again, in using the same lactalbumin in the same amount, we have obtained almost no growth at all. However, the rations differed in this respect—when protein-free milk was present we obtained growth, but when a vitamine extract, salt mixture, and starch were substituted for the protein-free milk, poor growth resulted. In other words, our results agreed with what McCollum and his associates claimed; *i.e.*, the protein-free milk was responsible for supplying the needed accessory

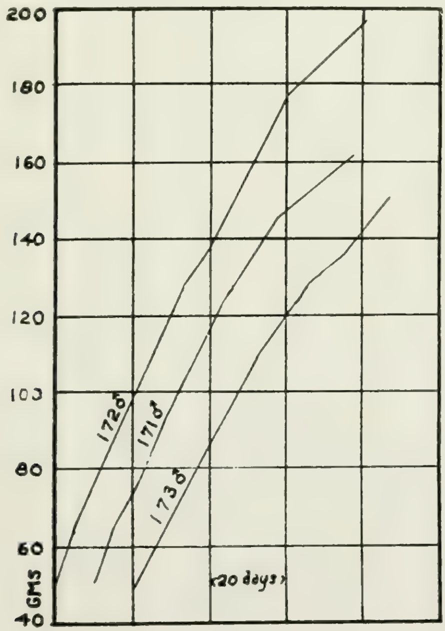

CHART 1. Good growth is obtained on a diet of 10 per cent of lactalbumin protein, 28 per cent of Osborne and Mendel protein-free milk, 18 per cent butter fat, 10 per cent lard, and starch.

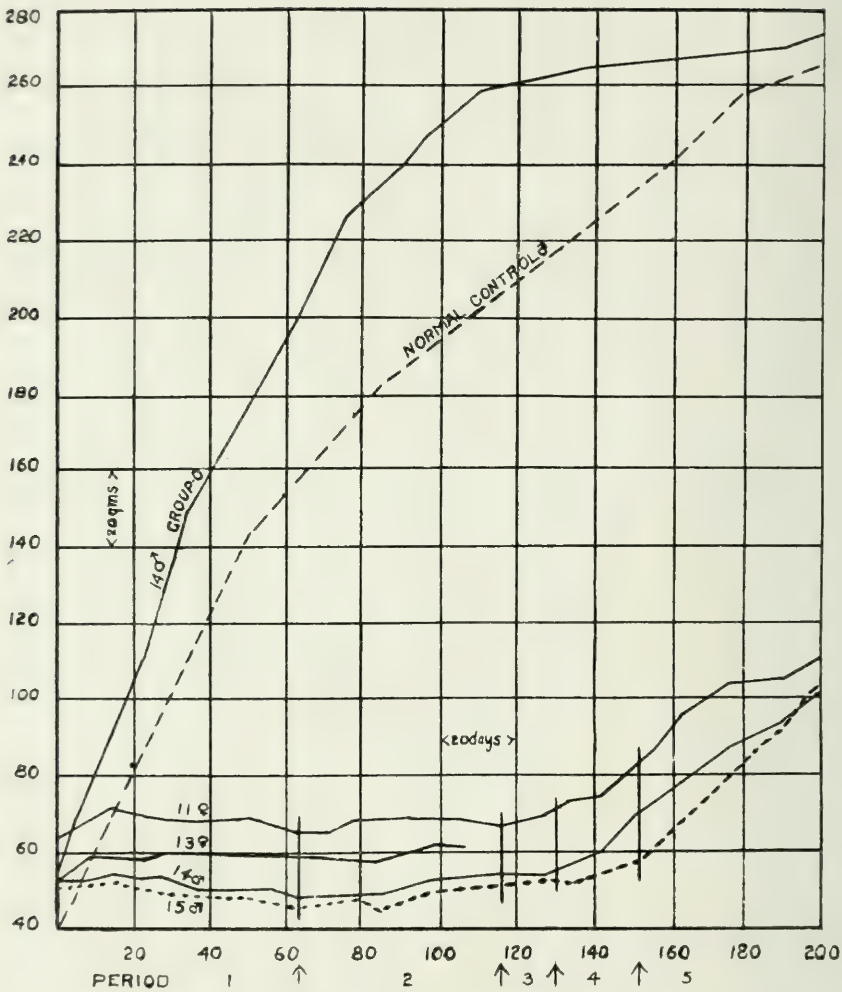

CHART 2. The curves illustrate in the case of Rat 14, Group O, the excellent growth response that can be obtained over a long period of time with the same diet that was fed to the groups of rats represented in Chart 1.

The curves also show, in the case of Rats 11, 13, 14, and 15, an interesting cycle of growth. Thus, throughout, the basal diet was 28 per cent of the Osborne and Mendel protein-free milk, 18 per cent butter fat, 10 per cent lard, and starch to make up the balance. In Period 1 (maintenance) there was 7 per cent corn gluten protein; in Period 2 (maintenance) 10 per cent corn gluten protein; in Period 3 (slight growth) 7 per cent protein, of which 28 per cent was lactalbumin and 72 per cent corn gluten; in Period 4 (growth) 10 per cent protein with 18 per cent of it lactalbumin and 82 per cent corn gluten; and in Period 5 (growth) 10 per cent protein, of which 32 per cent was lactalbumin and 68 per cent corn gluten. There can be no doubt that the lactalbumin is capable of supplementing the growth-deficient corn gluten protein.

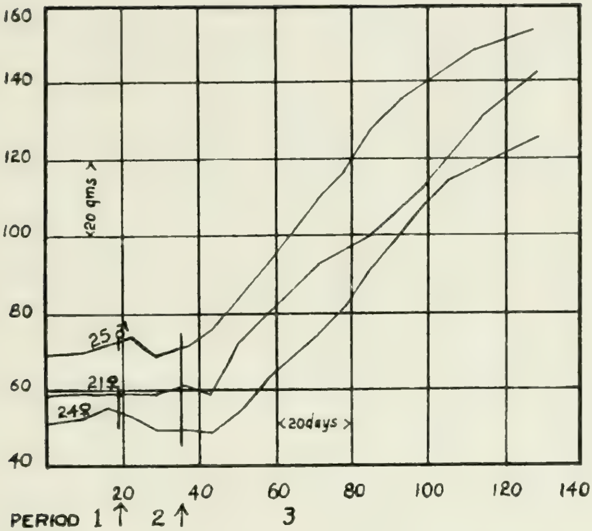

CHART 3. The curves illustrate the effect of incorporating the Mitchell and Nelson protein-free milk in place of the Osborne and Mendel product—the former being lower in protein nitrogen. Throughout, this protein-free milk was present. In Period 1 (maintenance) there was 7 per cent corn gluten protein; in Period 2, 6.5 per cent protein, of which 22 per cent was lactalbumin and 78 per cent corn gluten; and in Period 3, 8 per cent protein, of which 36 was lactalbumin and 64 per cent corn gluten. It will be seen that good growth took place in Period 3, when the lactalbumin supplemented the corn gluten to the extent of 36 per cent with the Mitchell and Nelson protein-free milk present.

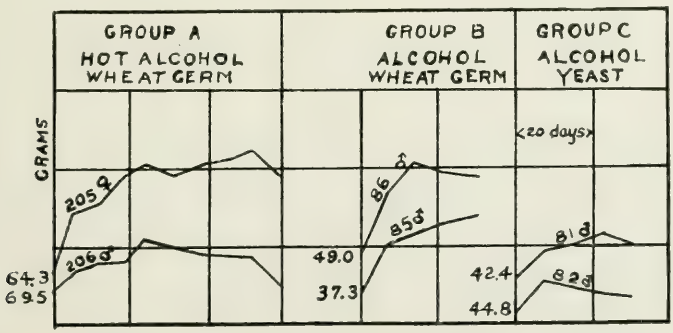

CHART 4. The curves illustrate the poor growth obtained with lactalbumin as the sole protein when protein-free milk was not used. The diet was made up of 10 per cent lactalbumin protein, 3.7 per cent salt mixture 185 (McCollum), 18 per cent butter fat, 10 per cent lard, 5 per cent of the hot alcohol extract of ether-extracted wheat germ or 1 per cent of the cold alcohol extract of dried brewers' yeast, and starch to make up the balance.

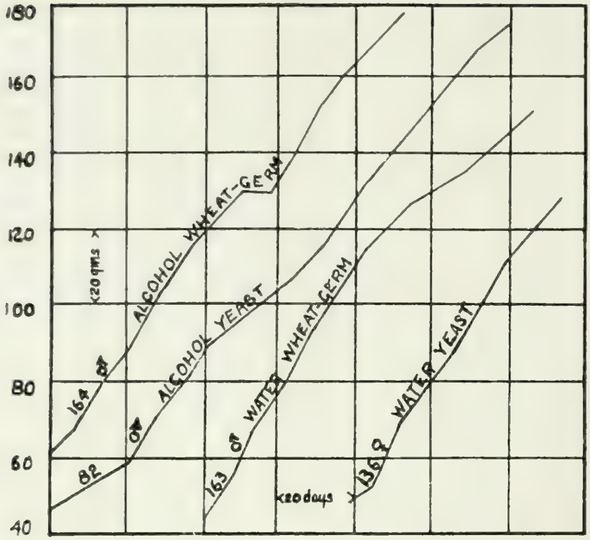

CHART 5. The rations fed these groups of rats were composed of 10 per cent lactalbumin protein, 18 per cent butter fat, 10 per cent lard, 3.7 per cent salt mixture 185 (McCollum), 24.6 per cent purified lactose, water and alcohol extracts of either wheat germ or yeast to supply the water-soluble B vitamin, and starch to complete the diet. Normal growth was possible with each of these four diets even in the absence of protein-free milk.

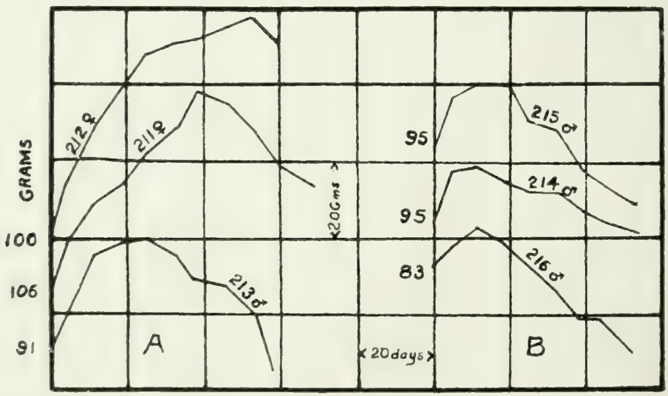

CHART 6. In these rations, the purified lactose was incorporated as the sole carrier of the water-soluble B vitamin. The diet for Group A was butter fat 5 per cent, lactalbumin protein 10 per cent, purified lactose 20 per cent, salt mixture 185 (McCollum) 3.7 per cent, and starch. The diet for Group B was the same as for Group A except that it contained less starch and more fat, butter fat 18 per cent and lard 10 per cent. The curves indicate that both the low and high fat diets are deficient—slight growth occurring at the start which is soon followed by a cessation of growth and subsequent decline.

for the lactalbumin. *Our results do not show that the nitrogenous matter in this protein-free milk played the fundamental part in furnishing the necessary supplement.*

According to Osborne and Mendel, the protein of their protein-free milk constituted about 0.13 per cent of the food. Mitchell and Nelson claimed that the reduction in nitrogen which they were able to effect was due to protein. If this is so, their procedure gave a still lower protein content than that of Osborne and Mendel, bringing it down to about 0.08 per cent of the ration. Such small amounts of protein as these would hardly seem to be sufficient to supply enough cleavage products to cause the difference that McCollum and his associates found between casein and lactalbumin, or that we have found (Charts 1 and 4). Our lactalbumin was separated out, in accordance with the method of Osborne and Mendel. It was, however, thoroughly digested with hot alcohol a number of times, and finally with ether or acetone. In this way, we considered that all traces of the water-soluble B and fat-soluble A vitamins were removed.

With the idea of determining whether this growth-promoting accessory factor was specific for the protein-free milk when lactalbumin was the sole protein, various rations were fed which differed only in respect to the kind of synthetic protein-free milk that was used. These were made up of the McCollum (8) salt mixture 185, an equivalent amount of lactose that had been purified by repeatedly extracting a pure product with hot 95 per cent alcohol and drying in vacuum at a low temperature, purified starch, and an extract containing the water-soluble B vitamin. The extracts used were: a cold water digest of wheat germ; a hot alcohol (95 per cent) digest of wheat germ that had been previously extracted with ether to remove the oil; a cold alcohol (95 per cent) digest of dried brewers' yeast; or a water-soluble fraction of autolyzed brewers' yeast.

Chart 5 illustrates the growth-promoting property of these rations where lactalbumin was the sole protein and no protein-free milk was present, and shows that normal growth is possible. In the case of the water extract of wheat germ, which carried the vitamin B, the amount of solids used was

based upon the nitrogen content, 5 per cent yielding 0.11 gm. of nitrogen per 100 gm. of ration. The hot alcohol (95 per cent) extract of the wheat germ carried 0.08 gm. of nitrogen per 100 gm. of ration. And the cold alcohol extract of dried brewers' yeast added 0.04 gm. of nitrogen per 100 gm. of ration. In the case of the hot alcohol extract of wheat germ and the cold alcohol extract of dried yeast, the amount of protein nitrogen must have been very low, if in fact any was present. Therefore, the amount of nitrogen that these two extracts added to the rations was in the non-protein form.

It would seem then, if lactalbumin was in reality an incomplete protein for growth, that the various extracts, fur-

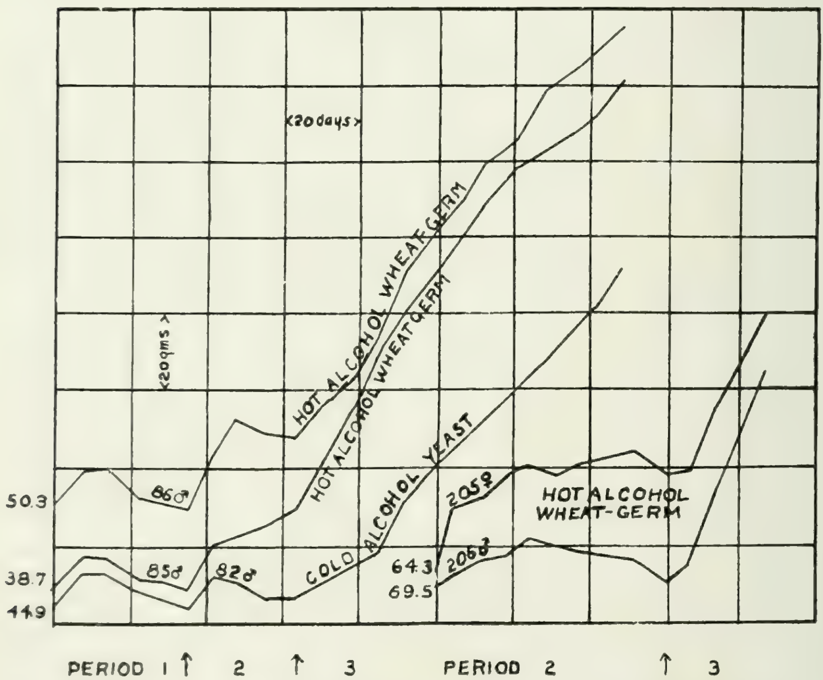

CHART 7. The curves show that lactose is an essential factor in the diet when lactalbumin is the sole protein. The basal ration throughout was lactalbumin protein 10 per cent, salt mixture 185 (McCollum) 3.7 per cent, butter fat 18 per cent, lard 10 per cent, and starch. The diet in Period 1 contained no water-soluble B accessory; in Period 2, this vitamin was supplied by an alcohol extract of wheat germ or yeast; in Period 3, 24.6 per cent of purified lactose replaced part of the starch of the ration used in Period 2. The growth response which followed the introduction of the purified lactose shows very definitely the importance of this constituent in these diets.

nishing the water-soluble B vitamin, carried the same non-protein cleavage accessory that the protein-free milk supplied. Further, the protein nitrogen, in the protein-free milk and in the water extract of wheat germ, was not concerned in supplementing the growth-promoting property of the lactalbumin.

McCollum and Davis (9) found that lactose and casein, unless thoroughly purified, seemed to carry enough water-soluble B and fat-soluble A accessories or vitamins to cause fairly good growth when these nutrients were added to a diet that was otherwise complete, except for these food hormones. Drummond (10) has confirmed their work. It might be contended, therefore, that these two accessory substances were absorbed by our purified lactose and that they were responsible for the growth which we obtained with the lactalbumin supplemented with the extracts of wheat germ and yeast.

Since both Osborne and Mendel, and McCollum and his associates used butter fat as their source of the fat-soluble A, we would be concerned only as to whether the *kind* or *source* of water-soluble B in the lactose was enough different from that in the wheat germ or yeast to be the contributing factor. The curves in Chart 6 show clearly that our lactose lacked a sufficient amount of the water-soluble B vitamin to promote growth. Thus, when this lactose and the lactalbumin were incorporated in rations, made up according to the Osborne and Mendel (18 per cent butter fat and 10 per cent lard) and the McCollum (5 per cent butter fat) formulas, the rats soon stopped growing and showed the usual symptoms which accompany a lack of this accessory in the diet.

As stated above, we have used rations which contained lactalbumin as the sole protein and which we considered from all the known facts obtainable to be abundantly capable of producing normal growth in rats. Yet some of these food mixtures gave poor growth (Chart 4), just as McCollum, Simmonds, and Parsons found with lactalbumin in their modified diet of peas, and as Osborne and Mendel (11) found when they compared the value of casein, lactalbumin, and edestin supplemented with their artificial protein-free milk and dried yeast. In these cases we did not use protein-free milk or lac-

tose. When the rations were modified so that a part of the starch was replaced by an amount of purified lactose equivalent to that present in 28 per cent of protein-free milk, the rats began to grow normally (Chart 7).

From the evidence we have offered, it would appear that the reason we were unable, in some cases (Chart 4), to obtain anything like good growth with lactalbumin as the sole protein, was due to the absence of lactose in the diets. Aside from this fact, it seems that the protein-free milk (which contained 80 per cent lactose) furnished nothing essential that was not supplied by the alcoholic extract of wheat germ or yeast, together with the synthetic salt mixture and lactose. Therefore, we do not agree with McCollum, Simmonds and Parsons that the high nitrogen content of the protein-free milk contributed some cleavage product which adequately supplemented what they claimed to be incomplete lactalbumin. *Rather, we take the position that the lactalbumin is a complete protein in the sense that it is not lacking in any essential nitrogenous cleavage product for growth.*

We are inclined to express the following tentative explanations, based upon our data, as to why lactalbumin does not produce normal growth unless supplemented by lactose in the form that we used it. First, the lactalbumin protein molecule may be so constituted that it is easily susceptible to toxic or inhibitory substances, while the casein protein molecule may be more stable or resistant. On this basis we would say that in the wheat germ and yeast extracts, which carried the water-soluble B accessory, there may have been some toxic substance present which prevented normal growth with the lactalbumin until the lactose was introduced. With the modified lactalbumin-peas diet of McCollum, Simmonds, and Parsons (5), there was also present a toxic substance which acted exactly as it did in our diets when no lactose was present, but in the casein-peas diet the toxicity factor did not manifest itself.

Second, in the separation of the casein, the lactalbumin, and the protein-free milk from fresh skimmed milk, the lactose (and possibly the casein) adsorbs not only the water-soluble B and fat-soluble A accessories, as McCollum and

Davis and Drummond have shown, but it (and the casein) may *also* adsorb another as yet undefined water-soluble vitamin. In separating the lactalbumin, this accessory does not seem to be adsorbed, or if it is, the vitamin is split off much more easily during the subsequent washing and purification than is the case with lactose (or casein).

Applying this last suggestion, we would say that our purified lactose still had this undefined water-soluble vitamin adsorbed to it; on the other hand, that the casein of McCollum and associates was the carrier of this accessory; while in the feeding trials of Osborne and Mendel that this vitamine was present in their protein-free milk (2) but absent in their artificial protein-free milk without lactose (11). To substantiate this deduction regarding casein, we refer to the work of Funk and Macallum (12), who found that this protein, even though it was purified by the method which McCollum (9) uses, carried an accessory which they say may be analogous to the antiscorbutic vitamin.

It seems plausible to conclude from these data that the second explanation is the more probable, although it is easy to conceive that both the above suggestions may be needed at times to explain the nutritive value of the lactalbumin. It is, therefore, difficult, in the light of these results, to agree with McCollum, Simmonds, and Parsons that the protein-free milk carried enough *nitrogenous* cleavage products to supplement what they designate as the incomplete and deficient growth-promoting lactalbumin, and yet when this protein-free milk supplemented the casein, it contributed nothing to accelerate the rate of growth of this protein and make it even the equal of the lactalbumin. If their conclusion was correct, how can one explain why Osborne and Mendel (2) obtained such contrasting results as to the relative growth-promoting values of lactalbumin, casein, and edestin when these proteins were incorporated in rations with the same amount of protein-free milk present in each (13)?

CONCLUSIONS.

1. Lactalbumin is a complete protein in the sense that it does not lack any of the nitrogenous cleavage products essential for growth. It can supplement a deficient growth-pro-

moting protein (corn gluten) and, incorporated as the sole protein in a ration containing lactose, it produces a normal rate of growth, when present to the extent of only 10 per cent.

2. Lactalbumin is a protein which either is sensitive to certain toxic substances and under these conditions growth is retarded unless adequate adjustment is made in the diet; or it is a protein which does not appear to be able to adsorb what we tentatively designate as a vitamin (other than water-soluble B).

3. Lactose seems to be the essential constituent in the protein-free milk that contributes the accessory which makes an otherwise incomplete lactalbumin diet bring about normal growth in rats.

4. Lactose, when added to a lactalbumin diet, either may have the physiological property of overcoming the toxicity that inhibits growth taking place, or else it may carry a water-soluble vitamin (other than the water-soluble B) which appears to be essential to growth. This phase of the study is being continued.

The authors wish to express their appreciation to Lieutenant M. E. Slater for valuable assistance in the preliminary work of this study. He was obliged to sever his connection with this work at an early stage as he was called into military service. To Mr. Charles Hunter also our thanks are due for aiding in the routine work.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 204, 1919.

(Reprinted from the *Journal of Laboratory and Clinical Medicine*, Vol. 4, No. 8, May, 1919.)

SEDIMENTING SUSPENSION OF LIVE BACTERIA ON A LARGE SCALE.

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In the sedimentation of bacterial suspensions or broth cultures for the preparation of bacterial vaccines, or for the purpose of clarifying the broth before filtration in the process of obtaining toxins, where the question of time as well as quantity must be considered, laboratories have been compelled to resort to the use of a centrifugal machine run on the principle of a milk separator, such as the Sharples machine, which will permit of a continuous flow of liquid to and from the machine.

It has been found, however, that when the bacteria are being sedimented in a live state, it is rather an unsafe procedure for the operator unless some protection is afforded against the fine spray, containing bacteria, which is given off from the liquid as it leaves the rapidly revolving bowl.

It has also been observed that the chances of obtaining a sediment or a filtrate in an uncontaminated state are rather doubtful unless the entire mechanism can be thoroughly sterilized before and after operation.

In order to obtain conditions as safe as possible for those in close proximity to the machine, and maintain, at the same time, an aseptic operation throughout, an apparatus has been devised according to plans suggested by one of us (E.M.H.) which fulfills the necessary requirements without influencing in any way the efficiency of the separator.

The apparatus in question consists of a cast iron jacket, opening on the front, with a swing door, which completely encloses a Sharples turbine-driven laboratory centrifuge, including its supply can. The jacket is so constructed that it becomes an autoclave, when the door is securely shut and

the valves of the various pipes leading to the jacket are closed, in which the entire mechanism of the centrifuge can be sterilized with live steam at a temperature of 120° C. for any length of time deemed necessary. At the same time connec-

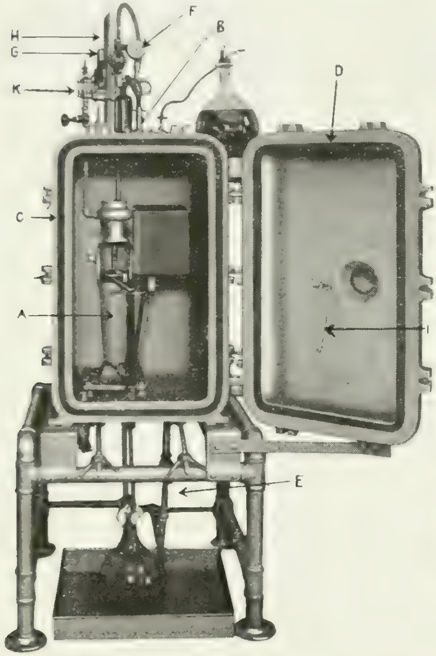

Fig. 1

A. Centrifuge.
B. Atomizer.
C. Cotton gasket.
D. Rubber gasket.
E. Pipe to vacuum system.
F. Air pressure gauge.
G. Air safety valve.
H. Discharge pipe to centrifuge.
I. Handle to supply can.
K. Oiler to centrifuge.

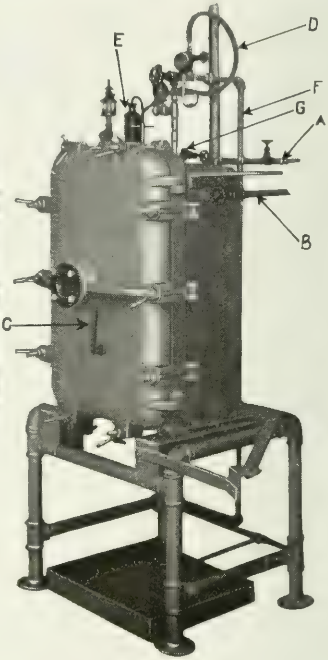

Fig. 2

A. Pipe to steam gauge.
B. Steam pipe.
C. Handle to supply can.
D. Air pipe to atomizer.
E. Bottle of germicide for atomizer.
F. Compressed air pipe.
G. Valve for siphon intake.

tion is maintained between the jacket and a vacuum system in order to facilitate the drying of the several parts of the machine, after the steam has been exhausted, without opening the door of the jacket.

To further insure the safety of the operator, in addition to a rubber gasket, on the inner surface of the door where it comes in contact with the rim of the jacket, is found a roll

of cotton, soaked in a germicide, packed in a groove surrounding the frame of the door, making the door crack entirely germ-proof.

The apparatus is also equipped with a safety valve for the compressed air system as well as an automatic steam pressure gauge to doubly safeguard both the operator and the machine.

If it is found necessary to gain access to the machine any time before the parts can be autoclaved, sterilization of all exposed surfaces may be accomplished by means of a large atomizer, connected with the compressed air pipe and a bottle of some strong germicide, which is placed in the top of the jacket. This atomizer is so constructed that it will revolve in a complete circle, in order to reach all sides and corners, and of such dimensions that it is capable of discharging with an air pressure of 30 to 50 pounds, causing the entire chamber to be filled with a fine cloud of spray and rendering any exposed parts perfectly safe to handle within a short space of time.

In order to control the flow of liquid from the supply can to the bowl, when the door is closed, a lever, having a handle on the outside of the door, is made to connect with the faucet of the supply can so that it can be manipulated at will without exposing the inside of the jacket.

The apparatus has been given a thorough trial for several months on various vaccines and toxins, and has been found perfectly efficient as to its qualifications as a sterilizer, as well as a separator.

In centrifugalizing a certain lot of material the procedure is as follows: Set up the machine for operation, clamp the door, shut all necessary valves, autoclave, and then dry with vacuum. Start machine, allow suspension to siphon from flask, on top of jacket, into supply can. From there it will flow into the bowl and thence out of the nozzle through the funnel into the plugged flask under the jacket. The sediment will be found in the bowl and the clear filtrate in the flask, both uncontaminated.

Studies from the Medical Research Laboratories, Parke, Davis & Co. Reprint No. 205, 1919.

(Reprint from *The Journal of Laboratory and Clinical Medicine*, Vol. IV, No. 8. May, 1919.)

ON THE DETECTION OF CHLORETONE IN MOTHER'S MILK.*

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It is a widely recognized fact that milk from lactating mothers may possess a foreign taste, odor or color following the ingestion of certain substances. As concrete examples, alcohol has been found,[1] likewise opium and morphine, zinc, iron, lead, arsenic, antimony, bismuth, and mercury. These facts become of utmost importance in reference to the administration to the mother of such drugs as may be injurious to the child.

The extensive use of chloretone therapeutically calls for further research in this particular on this important drug. The presence of chloretone in the tissues, organs and fluids of the body after ingestion has been conclusively demonstrated by Aldrich.[2]

The purpose of these experiments has been to determine the presence and amount, if any, of chloretone in the milk of lactating mothers. Humans and dogs were used as experimental subjects.

The method of demonstrating and estimating the presence and quantity of the drug was precisely that originated by Aldrich.[3] The efficacy of this method depends upon the characteristic crystalline form of chloretone, volatility with steam, and low solubility in water. A brief résumé of the method may not be out of place.

The material under investigation, properly comminuted or digested if necessary, is subjected to steam distillation. Great care should be exercised in keeping all connections absolutely tight. The distillate is placed in a flask, which is connected with an up-

*My thanks are due to Sister Olimpia and Miss Peltier of the Providence Hospital, Detroit, who so kindly co-operated with me in the matter of securing patients and rendering other valued assistance.

right condenser having a circulation of very cold water, and gently heated. If the test is positive, crystals of chloretone, long and feathery, will appear in the vicinity of the steam crest. A hand glass facilitates the detection in case of very small quantities. This test possesses much delicacy, as little as 1 m. or 1/66 gr. having been readily detected.

EXPERIMENTAL.

Case I.—Mrs. J. Ten gr. of chloretone were administered and after one hour 60 mils of milk were taken. Results negative.

Case II.—Mrs. A. One hour after the administration of 15 gr. of drug, 90 mils of milk were taken. Results negative.

Case III.—Mrs. S. One hour after the administration of twenty gr. of drug, 60 mils of milk were obtained. Results negative.

Case IV.—Mrs. E. Two hours after administration of 10 gr. of chloretone, 60 mils of milk were taken. Results negative.

Case V.—Mrs. McM. Two hours after administration of 15 gr. of drug, 75 mils of milk were obtained. Negative results.

Case VI.—Mrs. H. Two hours after administration of 20 gr. of drug, 50 mils of milk were taken. Negative results.

Case VII.—Mrs. B. Ten gr. of chloretone administered and 60 mils of milk taken three hours later. Results negative. Four hours from time of administration of drug, 30 mils were obtained. Negative results. Five hours later 40 mils. Negative results.

Case VIII.—Mrs. C. Twenty gr. chloretone administered and samples averaging 40 mils taken at the end of every hour until five were obtained. Results proved negative.

Case IX.—Mrs. D. Ten gr. of chloretone administered and one hour later 70 mils of milk obtained. Two hours from time of first administration 10 gr. more were given and one hour later 50 mils milk obtained. Two hours from time of second administration 10 gr. more were administered. One hour later 45 mils milk obtained. Two hours from third administration 10 gr. more were administered and 40 mils milk obtained. A total of 40 gr. chloretone had been administered in a space of eight hours. No positive test obtained.

Case X.—Mrs. De T. Exact duplicate of Case IX. Negative results.

Case XI.—Mrs. R. Fifteen gr. chloretone administered and one hour later 65 mils of milk removed. Two hours from time of first administration 15 gr. more were administered and one hour later 50 mils milk obtained. Two hours later 15 gr. more drug given and one hour later 40 mils milk obtained. A total of 45 gr. of drug given in a space of six hours all produced negative results.

The above mothers were normal women in every respect and were nursing their own infants throughout the period of experimentation. No effect on the infants was discernible; however, this would be very difficult as a normal infant sleeps the greater part of the day.

The following experiments were then performed on lactating dogs:

Case A.—Lactating dog weighing 10.5 kilos was obtained seven weeks after parturition. The young were taken away at beginning of experiment. Dog normal in every respect except very thin.

Three grams of chloretone were dissolved in 3 mils of 95 per cent alcohol and poured into 60 mils of tepid water.

This suspension was administered per mouth to dog fifteen minutes after complete removal of milk by young. Dog immediately passed into profound sleep and remained in such condition for four days. At no time before recovery or after could any milk be obtained.

These results pointed to the necessity of a change in method of administration of drug.

Case B.—Lactating dog, 10.5 kilos in weight, five weeks after parturition was taken from her young and milk completely removed by mechanical suction 15 minutes before administration of chloretone. The drug 0.5 gm. was dissolved in the least possible amount of alcohol and intimately mixed with a portion of chopped meat. Seven hours later 15 mils of milk were removed and .8 gm. of the drug was administered in precisely the same manner as before.

This portion of milk to be known as Sample 1 gave negative results.

Seventeen hours later 85 mils of milk were taken and .8 gm. of drug again given. Sample 2 also gave negative results.

Twenty-four hours later milk removed from dog as completely as possible. Amount obtained 65 mils. Dog rather drowsy at this time. One gm. of drug administered. Sample 3 tested very positive. Estimated about 2 mg.

Seven hours later milk again taken from dog. Amount obtained 15 mils. At this time only .6 gm. of chloretone given as dog had become very drowsy.

Sample 4 gave positive test.

The following morning, seventeen hours later, 30 mils of milk were obtained and 1 gm. of drug again administered. Positive test for Sample 5.

Seven hours later attempt was made to obtain more milk, but only about 2 mils were obtained. Administration of chloretone omitted this time. Stimulation, only, kept dog awake. Except for great sleepiness dog is normal.

The following morning milk was again removed. Seven mils were obtained. Chloretone 1 gm. again administered.

Sample 5, while positive, but barely perceptible, undoubtedly due to small amount of milk.

No drug was given in afternoon, neither was it possible to obtain any milk.

Seventeen hours later no milk could be obtained. Necessarily all experimentation came to an end.

The question here arises as to the effect of chloretone as an antigalactagogue. The results of the experiments on dogs point to such an effect, and this is in accordance with other findings in regard to chloretone as an inhibitor of the formation of bodily fluids. No such effect, however, was noticed in the case of human subjects. This undoubtedly is due to the small amount of the drug used, as weight for weight the dog received *in toto* twelve times as much chloretone as any human subject.

CONCLUSIONS.

Chloretone is found in the milk only upon continuous administration of very large doses.

Upon the administration of 5 to 20 gr., the usual therapeutic dose, no trace is found even though administered several times in succession.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 206, 1919.

(Reprinted from the *American Journal of Veterinary Medicine,* Vol. 14, No. 5, May, 1919.)

AN OUTBREAK OF HEMORRHAGIC SEPTICEMIA AMONG SHEEP.

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Hemorrhagic septicemia in sheep has been reported, both in Europe and in the United States, on several occasions. Baker (1) states that the disease is rare in this country. The disease has made its appearance in a number of widely separated localities within recent years, especially in some of the States west of the Mississippi River. Ward and Beebe (2), of Minnesota, reported an outbreak of the disease among sheep in 1911. Glover, Newsom and Alkire (3) recently reported the existence of the disease in Colorado. Several veterinarians have reported the disease to be rather prevalent in the Northwest. The disease designated as "septicemia pluriformis ovium," by Miessner and Schern (4), is apparently the same pathological entity. M'Gowan and Rettie (5) have reported upon the disease in Scotland, and these authors are of the opinion that many of the cases of so-called "braxy" are probably hemorrhagic septicemia. Recently Mori (6) published his observations on the disease in Italy.

Just as we have variations in the clinical manifestations of hemorrhagic septicemia in other species, more especially among cattle, the disease in sheep seems to be subject to the same influences, and in different outbreaks of the disease these variations occur, frequently confusing the clinician who has not had a great deal of experience with the disease. There are certain sections of the United States where the disease is apparently unknown in any form or in any species. There are also localities where cases of the disease have been met rather frequently among cattle, to the exclusion of other species. Of course, it is quite possible that the disease has been overlooked or failed to be recognized in other species.

THE PRESENT OUTBREAK.

In a paper to be published later, the author will discuss the relationship between hemorrhagic septicemia and the disease of young cattle known variously as "stock-yards fever," "stock-yards pneumonia," etc., which has been rather prevalent during recent years. The most constant feature of the disease is its predilection for young cattle of the stocker and feeder class, which have passed through certain of our large public stock-yards. In the outbreak to be described the sheep were not natives, but had recently been shipped into southern Michigan, via Chicago, the band having originated in Montana.

On October 10, 1916, a trainload of sheep arrived at Manchester, Mich. There were in the neighborhood of 9,000 sheep in the shipment, consisting of 29 double-decked carloads, about 300 sheep to the car. These sheep were distributed among some thirty different farmers, around Tecumseh and Adrian, for feeding purposes. They were driven from Manchester to their respective destinations on the first, second, third and fourth days following their arrival in Manchester. The purchasers of the sheep were of the opinion that the animals were in very good condition, considering their long journey, which had been made in quicker time than usual.

Sickness made its first appearance among the sheep just ten days after their arrival at destination. In a few instances the new sheep had mingled with native sheep, but inquiry failed to elicit information of the spread of the disease to the native sheep. Although practically every farmer who purchased some of the sheep reported losses, the percentage of the losses on different farms varied considerably. One man lost 45 out of 350, while his neighbor lost only four out of a carload. Another man lost 25 out of two carloads. At first it was believed that the sheep were dying from digestive disturbance following change of feed. This idea was abandoned when it was discovered that there was sickness in practically every flock, regardless of the kind of feed or method of feeding, which was quite variable among the thirty farmers. Some of these men were experienced sheep feeders, and had handled sheep successfully for many years. The suggestion that parasites were the cause of the trouble was soon dismissed, following observations of the clinical symptoms and a few post-mortems.

The symptoms were rather uniform in most cases. Temperatures ranged up to 105° F. Many animals were completely off feed. Depression was very noticeable, many of the animals standing around with backs arched. Breathing was accelerated. Some of the sheep exhibited colicky pains and were very restless and uneasy at times. Coughing and sneezing occurred quite frequently. Some of the sheep ground their teeth persistently, and muscular tremors were much in evidence. There was cessation of rumination in most cases. One of the most marked and constant symptoms was the passage from the bowels of a thin, blood-stained discharge, having a very fetid odor in most cases. The animals were usually sick for a few days before death supervened, and several animals which exhibited only mild symptoms made a recovery.

Upon post-mortem examination the lesion most frequently met with was a very severe hemorrhagic enteritis, most marked in the small intestine. Frequently the cecum was extensively involved. Petechial hemorrhages were noted on the heart muscle, and areas of lobular pneumonia were noted quite regularly in the lungs. A marked distention of the gall-bladder was noted in one case autopsied. Intestinal parasites were found in some cases, but in others none were found.

Samples of fresh tissues and heart blood were brought to the laboratory, in two sets, three days apart. The first lot consisted of the hearts of two sheep, wrapped in gauze wet with an antiseptic, which were brought to the laboratory by the first veterinarian called to see the sheep. The bacteriological examination of these tissues was negative, no growth being obtained in plain bouillon, plain agar and blood agar. Direct examination of smear preparations failed to reveal any suspicious organisms. In spite of the negative outcome of the bacteriological examination, a tentative diagnosis of hemorrhagic septicemia was made, based on the history of the outbreak and the symptoms shown by the sheep, and the author proceeded to the scene of the outbreak for the purpose of making further observations and securing additional material for laboratory examination.

The second lot of specimens consisted of heart blood from two animals, one that had died just previous to autopsy, and the other killed in a moribund condition. One heart with the vessels ligated

was also brought to the laboratory. From the coronary vein of the heart a small amount of blood was secured and used to inoculate culture media. The two samples of heart blood were also used to inoculate various media. It is quite interesting to note that the media inoculated with the blood secured from the heart chambers remained sterile, while the cultures made from the coronary vein yielded an organism which was later positively identified as bacillus ovisepticus. The isolation and identification of the hemorrhagic septicemia group of organisms will be discussed in a future paper on this subject.

TREATMENT.

Very little treatment was applied in this outbreak. The disease seemed to be at its height when the author made his first visit, and some of the sheep were believed by the owners to be recovering. Very few new cases developed during the seventy-two hours consumed in getting the materials to the laboratory, isolating and identifying the organism. As soon as an animal was noticed to be sick, it was isolated. Kreso was added to all drinking water, and everything done to improve the hygienic conditions.

DISCUSSION.

Attention has already been called to the similarity of the present outbreak of ovine hemorrhagic septicemia and so-called "stockyards pneumonia" of young cattle, from the epidemiological standpoint. Investigations of the latter disease have already very strongly suggested the etiological relationship of bacillus bovisepticus to the cattle disease. In the present outbreak we have a history, certain clinical manifestations, post-mortem pictures and laboratory findings which fit in very nicely as links in the chain of evidence to prove that the cattle and sheep diseases are similar in nature, and that the hemorrhagic septicemia organism plays some important role in the etiology of the disease.

Sporadic outbreaks of ovine hemorrhagic septicemia are met with, just as such outbreaks sometimes occur among cattle, without the history of recently having passed through a public stockyards. Usually such cases are acute, deaths occur very suddenly, often without warning, and it is no unusual experience to have

such outbreaks cease just as suddenly as they started. Atypical cases present difficulties in the way of making a prompt and accurate diagnosis clinically. The characteristic petechial hemorrhages which for so long have been associated with the disease can hardly be said to be absolutely specific for hemorrhagic septicemia. These lesions are frequently seen in other pathological conditions. Failure to isolate the hemorrhagic septicemia organism from a given case or cases of the disease is not evidence *per se* of the non-existence of the infection. On the other hand, there is even some doubt as to whether we are perfectly safe in making a diagnosis of hemorrhagic septicemia simply by demonstrating the presence of bipolar organisms in the blood or tissues of a dead animal.

SUMMARY.

Hemorrhagic septicemia is a serious disease of sheep. It has been encountered in widely separated localities, both in Europe and the United States. Either the disease is on the increase, or we have had it with us for some time and its exact nature was not previously determined.

In the outbreak of the disease here reported, wherein the disease made its appearance among a band of nine thousand sheep, shipped from Montana to Michigan, via Chicago, and distributed to some thirty farms on arrival, every one of the thirty flocks suffered losses, although the mortality varied widely on the different farms.

The similarity is pointed out, existing between this disease and that reported form of hemorrhagic septicemia in cattle more generally known as "stock-yards pneumonia."

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 207, 1919.

(Reprinted from *Rhodora, Journal of the New England Botanical Club*, Vol. xxi. 1919.)

NECESSARY CHANGES IN BOTANICAL NOMENCLATURE.

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POPULUS BALSAMIFERA Linn. Sp. Pl. ii, 1034 (1753); Miller, Dict. ed. 8, no. 5 (1768). *P. angulatus* Ait. Hort. iii, 407 (1789). —*Populus balsamifera* Miller, l. c., is referred by the Index Kewensis to *P. deltoides* and to *P. heterophylla*. A careful comparison of Miller's description with that of Linnaeus shows, however, that the two are identical, Miller having copied the technical description of Linnaeus, l. c., *verbatim*. Both quote Hort. Cliff., 460. A reference to the latter publication shows that species No. 4 *Populus foliis cordatis crenatis* is the one referred to. This is founded solely on *Populus nigra, folio maximo, gemmis balsamum odoratissimum fundentibus* Catesby, Car. i, 34, t. 34 (1731), a Carolina species, also quoted by Miller, l. c. There is therefore no question as to the identity of the one with the other and that the binomial *P. balsamifera* belongs to the Carolina Poplar, as usually understood, since in last analysis the Linnaean species is founded upon that of Catesby.

P. TACAMAHACCA Miller, Dict. ed. 8, no. 6 (1768). *P. balsamifera* Marshall, Arbust. Amer. 107 (1785), & French ed. 173 (1788). *P. viminea* Marsh. l. c. *P. candicans* Ait. Hort. iii, 406 (1789). *P. ontariensis* Desf. Hort. Par. *P. balsamifera* var. *candicans* A. Gray, Man. ed. 2, 419 (1858).—This is the common Balm of Gilead. Miller's name, which is the oldest, should be adopted for it instead of the later one of Aiton. A form with very scanty pubescense is

Var. **Michauxii** (Henry), n. comb. (*P. balsamifera* var. *Michauxii* Henry.)

Another form, generally without cordate leaves and pubescence, is the Northern Balsam Poplar that has so generally been

known as *P. balsamifera*. In accordance with priority this should bear the name

Var. **Lanceolata** (Marsh.), n. comb. (*P. balsamifera* Linn. Syst. Nat. ed. 13, ii, 656 (1770), and possibly of some earlier editions, and also of most subsequent authors, but not of Linn Sp. Pl. ii, 1034 (1753). *P. balsamifera* var. *lanceolata* Marsh. Arbust. Amer. 108 (1785), & French ed. 173 (1788).

Veronica persica Poir. Dict. viii, 542 (1808). *V. Buxbaumii* Ten. Fl. Nap. i, 7, t. 1 (1811).—*Veronica Tournefortii* C. C. Gmelin, Fl. Bad. i, 39 (1805), is the name employed in our local manuals to designate the plant that has, at times, been passing under the names of *V. Buxbaumii* or *V. byzantina*. A reference to Gmelin's Flora Baden shows: (1) that the specific name is based upon Tournefort's *Veronica orientalis, foliis Hederæ terrestris, flore magno*; (2) that the synonymy quoted is the Tournefortian species just mentioned, *V. filiformis* Sm. Trans. Linn. Soc. i, 195 (1791), and Buxbaum, Plantae minus cognitae Cent. i, t. 40, f. 1 (1727), all these being identical; (3) that the greater part of the description applies to *V. filiformis* Sm. The description of the leaf is that of *V. Buxbaumii*. While it is more than probable that Gmelin intended to include under his name both the species then known as *V. filiformis* Sm. and that which was later called *V. Buxbaumii* Ten., a careful analysis of all the factors to be considered can not leave any doubt but that he intended to make the Tournefortian species the type of his own, and this conclusion is particularly evidenced by the specific name itself, which certainly cannot have been derived from any other element. Since a binomial stands or falls with the element upon which it is founded, *V. Tournefortii* becomes a synonym of the older *V. filiformis* Sm. and its retention for *V. Buxbaumii* is erroneous. *V. persica* Poir. seems to be the oldest name applicable and should be taken up instead of *V. Tournefortii* for the species long known as *V. Buxbaumii*.

Viburnum Opulus Linn. var. americanum (Mill.) Ait. In *Rhodora*, xx, 14-15 (1918), Mr. S. F. Blake gives his reasons for dropping the "(Mill.)" from the authority for this variety and retaining "Ait." only. The reasons are that Miller's herbarium specimen of his *Viburnum americanum* is nothing more nor less than *Hydrangea arborescens* Linn. [Therefore by inference

Miller's species is a mere synonym of *Hydrangea arborescens* Linn.] and that as Aiton made no reference to Miller's publication, Miller should not be a part of the author-citation. The above argument of Mr. Blake is of the nature of a boomerang, for it is an excellent one against the practice he follows of adopting old herbarium specimens as types of species. Miller calls his species the Guelder Rose and says it has red berries, factors that will not permit of the species being referred as a synonym to *Hydrangea arborescens.* While Aiton did not mention Miller's publication there can be no doubt that he knew of it and that it was this knowledge that led him, when describing the plant as a variety, to use the same name that Miller did. The authority therefore should remain as heretofore "(Miller) Ait."

It would be interesting to know if Miller's *Hydrangea arborescens* is represented in his herbarium, and if so by a specimen of what species. Is it perhaps *Viburnum americanum*, thus proving another instance of the interchange of labels or of specimens? Philip Miller was too discriminating a botanist ever to have described in the same volume identical plants under two such widely diverse genera as *Hydrangea* and *Viburnum*.

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 208, 1919

(Reprinted from *The Journal of Bacteriology*, Vol. IV, No. 3, May 1919.)

STUDIES ON DIPHTHERIA TOXIN.*

II. THE ROLE OF THE AMINO ACIDS IN THE METABOLISM OF BACTERIUM DIPHTHERIÆ.

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Attempts to cultivate *Bact. diphtheriæ* in simple, non-protein media are recorded among the early studies with this organism. In 1892, Guinochet claimed to have grown diphtheria bacilli with toxin production in urine freed from albuminous constituents. Soon after, Uschinsky (1893) confirmed these findings and also reported the growth of *Bact. diphtheriæ* with elaboration of toxin in a medium having ammonium lactate and sodium asparaginate as the sole sources of nitrogen. The toxin thus obtained gave positive Millon's and xanthoproteic reactions, was precipitable by alcohol, and in its other reactions indicated protein characteristics.

The view that diphtheria toxin is a form of protein seems to have been first advocated by Brieger and Fraenkel (1890). These authors considered it to be a peculiar kind of albumin, which, because of its toxicity, they called "toxalbumin." Wasserman and Proskauer (1891), while conceding the possibility that pure diphtheria toxin may be an albuminous body, were of the opinion that the chemical reactions of the above "toxalbumin" were probably due to albumoses, present as impurities derived from bouillon. They also agreed with the observations of Brieger and Fraenkel that, because of its great susceptibility, this "toxalbumin" of diphtheria contained only a small portion of actual diphtheria toxin in an undecomposed state.

A unique view regarding the composition of diphtheria toxin

*Read before the Society of American Bacteriologists, Baltimore, Md., December 28, 1918.

was that advanced by Dzierzgowski and Rekowski (1892). From the fact that no diphtheria toxin was formed until the culture became alkaline, these investigators concluded that diphtheria toxin was a combination of certain bases and albumoses, of a nature similar to alkali albuminates.

Brieger and Boer (1896), working with both diphtheria and tetanus toxins, claimed to have isolated a double zinc-toxin compound, which showed neither albumin nor peptone reactions, thus arguing against the protein character of diphtheria toxin. In a subsequent communication regarding his synthetic diphtheria toxin, Uschinsky (1897) did not consider it of albuminous nature, although Chamberland filtrates of diphtheria bacilli grown in his medium gave distinct protein reactions. Not all cultures of *Bact. diphtheriæ* were found by him to be cultivable in a protein free medium, but when once accustomed to the medium, the organisms grew typically and as luxuriantly as in bouillon.

In an elaborate investigation comprehending seventy pure cultures of *Bact. diphtheriæ*, Hadley (1907) was unable to obtain any noticeable growth on Uschinsky's medium. Continuing the experimentation, this investigator finally devised a medium containing glycocoll and ammonium lactate together with glycerin, sodium chloride, calcium chloride, magnesium sulphate and di-potassium phosphate. This he found not only permitted the growth of diphtheria bacilli, but produced a culture which, after sixteen days' incubation, killed guinea pigs in from thirty-six to thirty-eight hours. Cultures of *Bact. diphtheriæ* which, at first, did not grow on the protein-free media, could be adapted to it by slow degrees, and the solid-staining forms of the organism always manifested the most rapid and luxuriant growth both during and after the adaptation. Hadley further concluded that, of the three nitrogen bases employed in the study, urea seemed to be of slight value, while glycocoll gave the best growth and strongest toxin. Asparagin appeared to give better results than the urea, although it was not as satisfactory as the glycocoll.

Hida (1908), in a paper concerning the significance of "peptone" in the formation of diphtheria toxin concludes that the deutero-albumose portion of the peptone is the most important for this purpose, while heteroalbumose, protalbumose, and amphopeptone are subordinate factors. A subsequent article by

the same author working with Teruuchi (1912-1913) also indicated that the production of toxin was influenced by the degree of decomposition of the "peptone." Most favorable results were furnished by a sample having 18 per cent of its total nitrogen in the monamino acid condition, 75 per cent precipitable by tannin, and 30 per cent by a 65 per cent concentration of alcohol.

The chemical composition of diphtheria bacilli has been reported upon by Tamura (1914), who examined, washed and dried pellicles of the organisms cultivated in a 2 per cent peptone, sheep-kidney extract medium. A monoaminomonophosphatid and a lipoid body were found to be present and the presence of adenin was considered as probable. Of the amino acids, arginin, *r* and *l* prolin, histidin, lysin, tyrosin, leucin, isoleucin, and valin were isolated, while tryptophane was demonstrated by reaction. On the other hand, no positive sulphur test could be obtained, and mycol was found to be absent.

More recently, Robinson and Rettger (1917, 1918) have reported upon the growth and toxin production of *Bact. diphtheriæ* when cultivated in a medium containing the product "opsine" previously studied by Dalimier and Lancereaux (1913). According to their description, "opsine is a biuret-free product resulting from the combined action of trypsin, erepsin and pepsin on certain protein materials not named by the authors or manufacturers." It was found to be abiuretic, and to give a high formol titration for monamino acids, the latter including leucin, tyrosin and very little tryptophane. In addition to the opsine, Robinson and Rettger attempted the cultivation of *Bact. diphtheriæ* in media composed of the protein-free acid hydrolysis products of casein, edestin, and lactalbumin.

Notwithstanding rapid and abundant growth of the organism, they found that very little diphtheria toxin was formed in the opsine, the minimum lethal dose being 0.2 per cent of the body weight. In the acid hydrolysis products media, the growth was considerably slower and less luxuriant than in the opsine and there was almost no evidence of any toxin. Addition of fresh beef infusion to opsine caused even more profuse growth of the bacilli than in diphtheria bouillon, while the toxicity was increased twenty-fold over that of cultures in opsine only. These authors consider that the results of their experiments tend to disprove the theory advanced by Uschinsky (1897) and also by

Hadley (1907) that diphtheria toxin can be directly synthesized from comparatively simple nitrogenous substances like the amino acids. On the contrary, they support the view "that more complex bodies, perhaps some of the proteoses, as claimed by Hida (1908), or polypeptids, are essential to the formation of toxin." What little toxin is found in protein-free media, according to these investigators, may be formed from the disintegration products of dead bacilli during the long period of incubation.

It will be readily conceded from the above résumé that comparatively little information is available regarding the constitution of diphtheria toxin. The two experimenters, Hadley and Uschinsky, who claim bacterial synthesis of the toxin, injected cultures of *Bact. diphtheriæ* grown in their non-protein media for determination of toxicogenicity. This technique is at once open to the criticism that, with diphtheria bacilli, pathogenicity toward susceptible animals is not necessarily an indication of toxicogenicity. It would seem more likely that the death of the test animals was produced by the living pathogenic organisms which survived the long incubation periods rather than by the disintegration products of dead bacilli as has been suggested by Robinson and Rettger.

Observations made by one of us, Davis (1917), in connection with a study of bacteriologic peptone, showed that, when incorporated in bouillon, the presence or absence of certain amino acids in the peptone had a decided influence on the growth of *Bact. diphtheriæ* and the production of its toxin.

The purpose of the work involved in the present paper was to determine, if possible, by cultivation of diphtheria bacilli in media consisting principally of known amino acids, the rôle played by these important protein constituents in the nutritional and toxicogenic requirements of this organism.

EXPERIMENTAL PROTOCOLS.

a. Preparation of materials.

With the exception of sodium asparaginate, which was obtainable in sufficient purity, the amino acids and nitrogenous bases employed in the experimental media later described were individually prepared and their identity established. These bodies comprehended glycocoll, cystin, glutaminic acid hydrochloride,

leucin, tyrosin, tryptophane, histidindichloride, glucoseamine hydrochloride, the purine bodies—hypoxanthin and xanthin and the extractives—creatin and creatinin.

Glycocoll was synthetically prepared, by the method of Nencki (1883), using powdered ammonium carbonate heated with monochloracetic acid. The glycocoll was isolated as the copper salt, decomposed with hydrogen sulphide, clarified with purified animal charcoal and crystallized. Several recrystallizations gave pure needles, answering all requirements for glycin.

Washed wool was found to be a very satisfactory raw material for cystin. The procedure given by Folin (1910) was followed with some modifications. The wool was hydrolized with hydrochloric acid, an excess of sodium acetate added, the precipitate washed, and then redissolved in boiling 5 per cent hydrochloric acid. After clarification with purified bone-black, an excess of ammonium hydroxide was added, the resultant phosphate precipitate removed, and the material precipitated by addition of acetic acid. Thorough washing and drying gave typical hexagonal plates of pure cystin.

Glutaminic acid was prepared as the hydrochloride from both gliadin and glutenin of wheat flour, according to the directions given by Osborne and Guest (1911). Hydrolysis was carried out with concentrated hydrochloric acid, the material decolorized, using in this case repurified blood charcoal, and the colorless filtrate finally concentrated to syrupy consistency in vacuum. On cooling for several days, a heavy crystalline deposit was obtained which was repurified from alcohol. The resultant product left no ash on ignition and showed all characteristics of glutaminic acid hydrochloride.

Both leucin and tyrosin were obtained from blood clots, using the separation method of Habermann and Ehrenfeld (1902). The clots were hydrolyzed with sulphuric acid (5:13), made alkaline with milk of lime, and the lime removed as calcium oxalate. The filtrate was then concentrated to obtain a maximum yield of crude crystals, and the leucin was separated from the tyrosin by boiling the crystals in a mixture of glacial acetic acid and alcohol. After hot filtration, the tyrosin was clarified with bone-black and recrystallized several times from cold water. Typical needles of tyrosin, giving a very strong Millon's reaction, were obtained. Additional quantities of this amino acid were

also derived from the preparation of tryptophane, as will be subsequently described.

The hot alcohol-acetic-acid filtrate containing the leucin was treated with an excess of copper carbonate, boiled, filtered, and the dark-blue filtrate concentrated to crystallization. The crystals were decomposed with hydrogen sulphide, the liquid decolorized with bone-black, and then again evaporated to the appearance of crystals. A number of recrystallizations from water furnished silver-like plates of pure leucin.

A modification of the method of Hopkins and Cole (1902) was employed for the preparation of tryptophane, using Bacteriologic Peptone (Parke, Davis & Company) as the source of raw material, which separated quantities of nearly pure tyrosin, requiring only crystallization to be suitable for use. The filtrate, after separation of tyrosin, was made 5 per cent acid with sulphuric acid and precipitation was effected with the mercuric sulphate reagent.

After thorough washing with sulphuric acid (5 per cent), the precipitate was decomposed with hydrogen sulphide and the latter removed with carbon dioxide. A partial precipitation with the mercuric sulphate reagent to remove cystin was now made, the precipitate removed, and an excess of the reagent added to the filtrate. The precipitate was again washed, decomposed with hydrogen sulphide, the latter removed with carbon dioxide, and whatever sulphuric acid was present was quantitatively removed with the requisite amount of barium hydroxide. Filtration gave a clear liquid, which was mixed with half its volume of 90 per cent alcohol, concentrated, decolorized with purified blood charcoal, and again concentrated in vacuum. After several crystallizations from alcohol, rhombic crystals of tryptophane were obtained giving an intense reaction with Benedict's (1909) modification of the glyoxilic acid reagent.

Blood clots were also found to be the most satisfactory source for the preparation of histidin. Essentially, the procedure given by Pauly (1904) was followed, the clots being hydrolyzed with concentrated hydrochloric acid. The hydrolysate was then concentrated, made only weakly acid with sodium carbonate, filtered, and then made distinctly alkaline with the soda. After freeing from ammonia and diluting, mercuric chloride was added to produce precipitation. The precipitate was thoroughly washed,

dissolved in hydrochloric acid (1:4) made alkaline with soda, and diluted, which caused the whole of the precipitate to be again thrown down.

Decomposition of the precipitate, after washing, was effected by hydrogen sulphide, the liquid was clarified with bone-black, filtered, and the filtrate evaporated to syrupy consistency. Equal volume of absolute alcohol was now added, and the material concentrated in vacuum. On cooling and recrystallizing small rhomboid crystals were obtained, giving an intense color with Pauly's (1940) reagent and answering all requirements for histidin dichloride.

The hydrochloride of glucoseamine was prepared by hydrolysis of chitin, obtained from decalcified lobster shells. Hydrochloric acid (1:2) was employed as the hydrolyzing agent, the liquid was clarified with blood charcoal and the filtrate evaporated to the appearance of crystals. The latter were recrystallized several times from water and finally were obtained pure in the form of parallelograms. In aqueous solutions, they strongly reduce Fehling's solution, have a distinct acid reaction to litmus and, on boiling with sodium hydroxide, are decomposed with elimination of ammonia.

Beef extract was used as raw material for hypoxanthin, xanthin and creatin, following the method given in Hawk's Practical Physiological Chemistry (1916.). Neutral lead acetate was added to precipitate the inorganic constituents, the excess of lead was removed with hydrogen sulphide, and the clear filtrate was concentrated to a thick syrup. After standing in the refrigerator for forty-eight hours, the syrup was extracted with 88 per cent alcohol, which left a residue of crystals. The latter were dissolved in water, decolorized with bone-black, the filtrate concentrated to small volume and allowed to crystallize. Repeated crystallization from water gave a substance which was identified as pure creatin. Creatinin was prepared from the creatin by hydrolyzing with dilute sulphuric acid. The excess of the latter was removed with barium carbonate, the material was filtered and evaporated to dryness. Extraction of the residue with 95 per cent alcohol and evaporation of the solvent gave pure creatinin.

The alcoholic filtrate from the above creatin preparation was evaporated to remove the alcohol, made ammoniacal, and pre-

cipitation effected by ammoniacal silver nitrate. The precipitate was now treated with boiling nitric acid (specific gravity of 1.1), filtered, and the hypoxanthine silver nitrate (identified by its crystalline appearance) was allowed to separate. The latter was decomposed by hydrogen sulphide, filtered, concentrated to remove the hydrogen sulphide, and make alkaline with ammonia. Free ammonia was removed by heating, the precipitate filtered, and the filtrate concentrated to small volume. On standing for several days in the refrigerator, small colorless needles of hypoxanthine were obtained.

To the filtrate from the hypoxanthine silver nitrate, ammonia was added in excess, the resultant precipitate filtered off and then decomposed with hydrogen sulphide. The filtrate was evaporated to a syrup and allowed to crystallize. Several recrystallizations gave pure xanthin.

The final reaction of all of the media described in the succeeding paragraph was adjusted to come within the limits, $C\overset{+}{H} = 1.0 \times 10^{-8}$ to $C\overset{+}{H} = 7.0 \times 10^{-9}$. This range has been found by Davis (1918) in a previous study on diphtheria toxin, to give very satisfactory results in the routine production of high potency toxin. It is important to note in this connection that it was found necessary to use the colorimetric method for accurate values of the media reaction. Electrometric determination of the hydrogen ion concentration of adjusted media containing amino acids proved to be unreliable, due to a steady increase in hydrogen ion concentration. In a typical case, the value rose steadily from an initial $C\overset{+}{H} = 9.5 \times 10^{-9}$ to about $C\overset{+}{H} = 2.5 \times 10^{-7}$ at the end of ten minutes.

Clark and Lubs (1917), in their work on hydrogen ion concentration, mention that synthetic media present some special problems in reaction and suggest the possibility of decomposition on sterilization. From the fact that the same phenomenon occurred on adjusting an unheated amino acid medium, the heat action can, in this instance, be left out of consideration. It is very likely that the increase is due to some action of the hydrogen while bubbling through the synthetic medium.

In all of the experimental work discussed below, the organism employed was a toxicogenic strain of *Bact. diphtheriæ* originally

obtained from Dr. W. H. Park and known as "Park No. 8." When grown in regular plain bouillon, under proper conditions, this culture has repeatedly furnished a toxin of which one L + dose is less than 0.15 cc. A twenty-four-hour culture in this medium shows a heavy pellicle and deposit with the intermediate bouillon entirely clear.

Unless otherwise noted, the general technic for toxicogenicity tests consisted in cultivating the organism in the experimental medium, contained in large flasks, for 14 days at 37° C. The purity of the culture was then checked, 0.4 per cent of purified cresols were added, the mixed material allowed to stand in the refrigerator for twenty-four hours, and then filtered through a Mandler filter. By this method a clear product was obtained for test in which any toxic effects due to bacterial protein were eliminated. The toxicity was determined by subcutaneous injection of 250 gram guinea-pigs in accordance with the usual procedure for valuation of the potency of diphtheria toxin.

b. Laboratory data.

1. *Synthetic media.* For preliminary experimentation looking toward the utilization of amino acids for the growth and toxin production of *Bact. diphtheriæ*, a number of synthetic media were prepared. The various ingredients comprising each preparation were dissolved in a stated volume of distilled water and heated in flowing steam for fifteen minutes. The requisite amount of 10 N NaOH was then added to give a final reaction in the completed product of $P\overset{+}{H} = 8.0$ to $P\overset{+}{H} = 8.2$. This was readily accomplished by cold titration against N/10 NaOH to a deep pink color with phenolphthalein, following the technic cited above (Davis, 1918).

The medium was now steamed again for fifteen minutes and the reaction checked colorimetrically (using phenolsulphonphthalein and standardized H_3BO_3 – KCl – NaOH solutions) as already discussed. After filtration and distribution as desired, the material was sterilized for twenty minutes at 115°C.

It was apparent, at once, that a *Bact. diphtheriæ* culture accustomed to peptone bouillon could not be successfully cultivated directly in synthetic media without preliminary acclimatization. Accordingly, successive transfers were made from the

bouillon into mixtures of bouillon and synthetic medium containing gradually increasing quantities of the synthetic media until finally what might be termed a "limiting concentration" of the synthetic medium was obtained. The various experimental media are discussed below in detail; and the general results are summarized in table 1.

Synthetic medium no. 1

CONSTITUENTS	AMOUNTS IN 1000 CC. OF DISTILLED WATER	CONSTITUENTS	AMOUNTS IN 1000 CC. OF DISTILLED WATER
	grams		*grams*
Tryptophane	0.30	Creatinin	0.10
Tyrosine	1.00	Sodium asparaginate	1.40
Leucine	3.00	Cystin	0.40
Glutaminicacidhydrochloride	1.60	Sodium chloride	4.00
Glycocoll	0.40	Dipotassium acid phosphate	3.00
Creatin	0.10		

Typical luxuriant growth was obtained in twenty-four hours with this medium in mixtures containing up to 96 per cent synthetic medium and 4 per cent bouillon. With concentrations of the synthetic medium greater than 96 per cent, there was a marked decrease in the luxuriancy of the growth and pellicle formation. Above 98 per cent of synthetic medium, practically no growth was discernible. Notwithstanding the heavy growth at 96 per cent, toxicogenicity tests made by the regular procedure at this concentration showed practically no toxin. Guinea-pigs receiving doses of 0.1 cc showed only irritation at the point of injection.

Synthetic medium no. 2.

CONSTITUENTS	AMOUNTS IN 1000 CC. OF DISTILLED WATER	CONSTITUENTS	AMOUNTS IN 1000 CC. OF DISTILLED WATER
	grams		*grams*
Tryptophane	0.40	Sodium asparaginate	1.20
Tyrosin	2.50	Histidindichloride	0.30
Leucin	2.50	Sodium chloride	4.00
Glutaminicacidhydrochloride	1.90	Dipotassium hydrogen phosphate	3.00
Glycocoll	0.85	Magnesium sulphate	0.50
Creatin	0.10	Potassium nitrate	0.20
Creatinin	0.10		
Cystin	0.40		

Very satisfactory growth but practically no toxin was obtained in mixtures containing 98 per cent of the above medium and 2 per cent of plain bouillon. With greater proportions of the synthetic medium the same effects were noted as with medium no. 1. Precipitation of the tyrosin in the medium under consideration showed an excess of this constituent. The following preparation was accordingly devised to contain a diminished quantity of this amino acid and also as a new factor, glucosaminehydrochloride, to note any increased action through carbohydrate addition.

Synthetic medium no. 3

CONSTITUENTS	AMOUNTS IN 1000 CC. OF DISTILLED WATER	CONSTITUENTS	AMOUNTS IN 1000 CC. OF DISTILLED WATER
	grams		*grams*
Tryptophane	0.60	Histidindichloride	0.50
Tyrosin	0.80	Glucosaminehydrochloride	1.50
Leucin	3.00	Sodium asparaginate	0.50
Glutaminicacidhydrochloride	2.50	Sodium chloride	2.50
Cystin	0.40	Dipotassium acid phosphate	3.00
Creatin	0.20	Magnesium sulphate	0.40
Glycocoll	0.80	Potassium nitrate	0.20

Practically the same results as regards growth and toxicogenicity were obtained with this preparation as with the preceding medium no. 2. The protocols of the foregoing experiments indicated that, in all probability, the preparation contained a number of superfluous constituents. Medium no. 4 was accordingly made up with only three amino acids and glucosaminehydrochloride, but with increased amounts of each of these substances.

Synthetic medium no 4

CONSTITUENTS	AMOUNTS IN 1000 CC OF DISTILLED WATER	CONSTITUENTS	AMOUNTS IN 1000 CC. OF DISTILLED WATER
	grams		*grams*
Tryptophane	1.25	Sodium chloride	2.50
Histidindichloride	1.25	Dipotassium acid phosphate	3.00
Cystin	0.42	Magnesium sulphate	0.40
Glucosaminehydrochloride	2.75		

Heavy typical growth was obtained in this case with 95 per cent of synthetic medium as the "limiting" concentration. With

a greater proportion of synthetic medium than 97 per cent, almost no growth was obtained. As was expected, the toxicogenicity results were negative.

Synthetic medium no. 5

CONSTITUENTS	AMOUNTS IN 1000 CC. OF DISTILLED WATER	CONSTITUENTS	AMOUNTS IN 1000 CC. OF DISTILLED WATER
	grams		*grams*
Tryptophane	0.60	Creatinin	0.08
Tyrosin	1.25	Xanthin	0.05
Leucin	3.00	Hypoxanthin	0.05
Glutaminicacidhydrochloride	2.50	Sodium chloride	4.00
Glycocoll	0.85	Dipotassium acid phosphate	3.00
Sodium asparaginate	1.00	Magnesium sulphate	0.50
Cystin	0.40	Glucose	1.50
Histidindichloride	0.50	Potassium nitrate	0.20
Creatin	0.20		

With this preparation, which was the most comprehensive synthetic medium investigated, heavy, typical growth was obtained in a mixture containing as high as 99.5 per cent of the synthetic and only 0.5 per cent of plain bouillon. A slight growth with slight pellicle formation was obtained even with 99.8 per cent of synthetic medium and only 0.2 per cent of plain bouillon, but with a concentration of bouillon less than the latter amount the growth failed.

It was deemed of interest, in connection with this medium, to determine what proportions of the synthetic medium and bouillon gave maximum toxin production. It was found that a toxin having an L+dose of 0.15 cc was still obtained where the mixture contained 90 per cent synthetic medium and 10 per cent bouillon. At the "limiting" concentration of 99.5 per cent, however, even with an apparently maximum growth, potency valuations showed practically no elaboration of toxin, which corroborates previous findings with synthetic media.

By way of possibly obtaining some information as to the important constituents in the above medium, a number of mixtures were prepared containing 99 per cent of differing synthetic media together with 1 per cent of plain bouillon. Inoculation of each was made directly from cultures grown in a 99 per cent

mixture of medium no. 5. The composition of the preparations is given below:

Synthetic medium no. 6

CONSTITUENTS	AMOUNTS IN 1000 CC. OF DISTILLED WATER	CONSTITUENTS	AMOUNTS IN 1000 CC. OF DISTILLED WATER
	grams		*grams*
Tryptophane	1.60	Sodium chloride	2.50
Cystin	0.40	Dipotassium acid phosphate	3.00
Glutaminicacidhydrochloride	2.50	Magnesium sulphate	0.40
Glucosaminehydrochloride	1.50		

The transfers into a 99 per cent mixture of the above showed only a scant growth, indicating this to be an inferior medium to no. 5 for cultivating *Bact. diphtheriæ.* On addition of sufficient bouillon to reduce the concentration of synthetic medium to 97 per cent, maximum intensity of growth was again restored.

Synthetic medium no. 7

CONSTITUENTS	AMOUNTS IN 1000 CC. OF DISTILLED WATER	CONSTITUENTS	AMOUNTS IN 1000 CC. OF DISTILLED WATER
	grams		*grams*
Tryptophane	1.00	Dipotassium acid phosphate	3.00
Cystin	0.75	Magnesium sulphate	0.40
Sodium chloride	2.50		

Almost no growth was obtained here in a 99 per cent mixture, and only very scant growth with the bouillon concentration raised to 5 per cent. A reduction of the synthetic medium concentration to 90 per cent was found to be necessary before luxuriant, typical growth could be obtained.

Synthetic medium no. 8

CONSTITUENTS	AMOUNTS IN 1000 CC. OF DISTILLED WATER	CONSTITUENTS	AMOUNTS IN 1000 CC. OF DISTILLED WATER
	grams		*grams*
Tryptophane	1.60	Histidindichloride	1.20
Cystin	0.40	Sodium chloride	2.50
Glutaminicacidhydrochloride	2.50	Dipotassium acid phosphate	3.00
Glucosaminehydrochloride	1.00	Magnesium sulphate	0.40

Heavy typical growth, comparable in character and intensity to that obtained in dilutions of medium no. 5, was obtained in 99 per cent mixtures of the above preparation. The luxuriance in this case, however, began to diminish at 99.2 per cent, and no growth was obtained in a mixture of 99.5 synthetic medium and 0.5 plain bouillon.

Medium no. 9. The fact that it was impossible to maintain metabolism of the microörganism in question on any of the preceding synthetic media alone, suggested that some important constituent might be lacking. By way of attacking the problem from a different angle, gelatin was hydrolyzed with 25 per cent sulphuric acid for twenty-four hours on the sand bath. Sufficient barium hydroxide was now added to make the hydrolysate distinctly alkaline and the resultant heavy precipitate was filtered off. The excess of barium hydroxide was next exactly neutralized with 10 per cent sulphuric acid, the precipitate removed and the clear filtrate (tested to prove absence of both Ba and SO_4 ions) was concentrated in vacuum to a thick syrup.

From the work of Levene and Beatty (1906), it appears that gelatin is lacking in cystin, tyrosine and tryptophane. To supply this deficiency in the above hydrolysate, the syrup was diluted with distilled water to a total solids concentration of 2 per cent and the following were added to each liter of medium:

CONSTITUENTS	AMOUNTS OF 1000 CC. OF DISTILLED WATER	CONSTITUENTS	AMOUNTS IN 1000 CC. OF DISTILLED WATER
	grams		*grams*
Tryptophane	0.40	Sodium chloride	5.00
Tyrosine	1.50	Dipotassium acid phosphate	3.00
Cystin	0.40	Magnesium sulphate	0.50

The resultant product was prepared, distributed, and sterilized as already described, and acclimatization studies were carried out as with the straight, synthetic media. Contrary to expectations, both the macroscopical appearance and the morphological characteristics of the organisms under investigation indicated that this medium was inferior to most of the preceding preparations. A mixture containing 90 per cent of the synthetic medium and 10 per cent of plain bouillon appears to be the "limiting" concentration permitting maximum growth and pellicle

formation. Even at this dilution of the medium, the growth is not as vigorous and the formation of pellicle appears more scanty than obtained in the unmodified preparations. Corroborating the foregoing, toxicogenicity results were negative.

TABLE 1

Growth and toxicogenicity of Bact. diphtheriae in synthetic media

MEDIUM NUMBER	MAXIMUM GROWTH CONCENTRATION		TOXICOGENICITY RESULTS	REMARKS
	Synthetic	Bouillon		
	per cent	*per cent*		
1	96.0	4.0	Negative	Organisms shorter, stain solid
2	98.0	2.0	Negative	Appearance more typical
3	98.0	2.0	Negative	Same appearance as no. 2
4	95.0	5.0	Negative	Growth not as heavy as preceding
5	99.5	0.5	Negative	Toxin having L+ dose = 0.15 cc obtained with 90 per cent synthetic, 10 per cent bouillon
6	97.0	3.0	Negative	Heavy growth light pellicle formation
7	90.0	10.0	Negative	Short, plump bacilli, stain solid
8	99.0	1.0	Negative	Heavy, typical growth and pellicle formation
9	92.0	8.0	Negative	Inferior growth scanty pellicle
10	90.0	10.0	Negative	Same as preceding

Medium no. 10. The fact that gelatin is of animal origin suggested the use for a culture medium of a vegetable protein hydrolysate. For this purpose, gliadin, the prolamine obtained from wheat, was prepared, according to the procedure given by Osborne and Clapp (1907). These investigators have shown that lysine is absent in gliadin, and that this protein contains only 0.02 per cent of glycocoll.

The gliadin was hydrolyzed exactly as carried out with the gelatin, using both the 25 per cent sulphuric acid and the barium hydroxide, and the final product, containing neither Ba nor SO_4 ions, was also diluted to a final total solids content of 2 per cent. Equal parts of this hydrolyzed product and of the finished medium no. 9 were then taken, 0.2 gram of tryptophane added per liter, the reaction adjusted, and this composite was then finished in the usual way. As will be noted from Table 1, practically the same results were obtained with this experimental preparation as with medium no. 9, and here, also, toxin production was unsuccessful.

2. *Elimination media.* It appears to be clearly indicated from the preceding experiments that metabolism of *Bact. diphtheriæ* is not permitted in a medium composed principally of amino acids. That the amino acids are, however, important factors in both growth and toxin elaboration with this organism seems to be plainly evidenced from the results already detailed with medium no. 5. The latter have also demonstrated that not all of these protein degradation products can exercise the same influence.

Preliminary cultivation in plain beef infusion carried out with the *Bact. diphtheriæ* strain employed above, showed that only a scant growth with no formation of toxin is possible in this medium. A Liebig's Extract of Beef solution gave even poorer results. More luxuriant growth but still no toxicogenicity occurred in a 2 per cent solution of peptone.

With the preceding facts in mind, several series, of what might be termed "elimination" media, were now devised having in each series one or two of the above three substances as basic ingredients. The individual amino acids or the other materials incorporated in the synthetic media already investigated were then added in the same quantities as previously employed. Adjustment of the reaction and finishing of these media were in accordance with the usual technic. In order to acclimatize the diphtheria culture to the various media, the organisms in each case were cultivated first for three successive generations in "starter" flasks containing 30 cc of the special broths and then inoculated into the large flasks.

The experimental preparations, together with the results obtained, are detailed in the accompanying table 2. As may be noted from the table, five series of the "elimination" media were prepared. The first set contained plain beef infusion as the basic ingredient, the second had 2 per cent peptone (Bacteriologic, Parke, Davis & Company) solution, and for the third series, a 1 per cent Liebig's Extract of Beef solution served as the common constituent. In the remaining two sets, ordinary bouillon (2 per cent peptone, beef infusion and 0.5 per cent of sodium chloride) and a meat extract bouillon (2 per cent peptone, 0.5 per cent salt, 1 per cent Liebig's Extract) were used as basic constituents.

The presence of any of these basic media in a preparation is

indicated in the table by a (*) and the absence of the others, by the symbol (—). The intensity of the growth has been characterized as follows: (++++) = heavy; (+++) = good; (++) = moderate; and (+) = scant. In the valuation of toxin potency, it was not deemed necessary to determine the minimum lethal dose (M. L. D.) above 1 cc, but where such results were obtained they have been designated by "M. L. D. = 1 cc +."

Probably the most significant of the data presented in table 2 are the results furnished with beef infusion alone, as a basic medium. Where 2 per cent peptone solution served as the common ingredient, practically no differences could be distinguished on addition of the special constituents. In each case, only the moderate growth due to peptone itself was obtained. Except where cystin was added, the results given with Liebig's Extract were negative.

The purpose in using regular (peptone beef infusion) bouillon and Liebig's Extract (peptone) bouillon as basic media was to determine whether addition of the amino acids and other special constituents caused any increase in potency of the toxin. As may be noted from table 2, the final toxicities furnished by the plain bouillon, special combinations are no higher than those given by the same culture of *Bact. diphtheriæ* when cultivated in the plain bouillon alone. Confirming expectations, the special media containing Liebig's Extract gave results uniformly inferior to beef infusion and, in no case, a final toxin of which one L + dose was less than 1 cc.

The addition of cystin to plain beef infusion, as may be seen from table 2, not only permits heavy typical growth with *Bact. diphtheriæ*, but a relatively strong toxin is also elaborated. Tryptophane, similarly, allows a heavy growth, but the resultant toxin is not as active as obtained with cystin. Practically the same results were obtained on adding either glutaminic acid hydrochloride or sodium asparaginate to the beef infusion. *Bact. diphtheriæ* grew luxuriantly in both cases, yielding a final toxin, however, which had only one-fifth the strength of that obtained with tryptophane. The growth in presence of glycocoll, it will be noted, was not as heavy as with the two preceding constituents, and the filtered toxin was also weaker (minimum lethal dose equals 0.77 cc).

TABLE 2

Effect of special constituents on growth and toxicogenicity of Bact. diphtheriae

CONCENTRATION OF SPECIAL CONSTITUENTS IN 1000 cc.	BASIC MEDIA			CHARACTER OF GROWTH	POTENCY OF TOXIN	REMARKS
	Beef infusion	2 per cent peptone	1 per cent Liebig's extract			
Tryptophane, 0.60 gram	*	—	—	++++	M. L. D. = 0.01 cc.	Strong growth with beef infusion base. L + dose of toxin = 0.75 cc.
	—	*	—	++	M. L. D. = 1 cc. +	
	—	—	*	+	M. L. D. = 1 cc. +	
	*	*	—	++++	L + = 0.15 cc.	
	—	*	*	+++	L + = 1 cc.	
Cystin, 0.50 gram	*	—	—	++++	M. L. D. = 0.0075	L + dose of toxin with been infufusion = 0.5 cc.
	—	*	—	++	M. L. D. = 1 cc.	
	—	—	*	+++	M. L. D = 1 cc.	
	*	*	—	++++	L + = 0.15 cc.	
	—	*	*	+++	L + = 1 cc.	
Tyrosin, 1.25 grams	*	—	—	++	M. L. D. = 1 cc. +	Only moderate growth with beef infusion base
	—	*	—	++	M. L. D. = 1 cc. +	
	—	—	*	+	M. L. D. = 1 cc. +	
	*	*	—	++++	L + = 0.15 cc.	
	—	*	*	+++	L + = 1 cc.	
Glutaminic acid hydrochloride, 2.50 grams	*	—	—	++++	M. L. D. = 0.05 cc.	Growth with beef infusion not as heavy as with cystin
	—	*	—	++	M. L. D. = 1 cc. +	
	—	—	*	+	M. L. D. = 1 cc. +	
	*	*	—	++++	L + = 0.15 cc.	
	—	*	*	+++	L + = 1 cc.	
Histidin dichloride, 0.50 gram	*	—	—	+++	M. L. D. = 1 cc. +	Growth with beef infusion base comparable to tyrosine
	—	*	—	++	M. L. D. = 1 cc. +	
	—	—	*	++	M. L. D. = 1 cc. +	
	*	*	—	++++	L + = 0.15 cc.	
	—	*	*	+++	L + = 1 cc.	
Leucin, 3.00 grams	*	—	—	++	M. L. D. = 1 cc. +	Moderate growth only with beef infusion base
	—	*	—	++	M. L. D. = 1 cc +	
	—	—	*	+	M. L. D. = 1 cc. +	
	*	*	—	++++	L + = 0.15 cc	
	—	*	*	+++	L + = 1 cc.	
Glycocoll, 0.75 grams	*	—	—	++++	M. L. D. = 0.07 cc.	Beef infusion growth not as heavy as with glutacid HCl
	—	*	—	++	M. L. D. = 1 cc. +	
	—	—	*	+	M. L. D. = 1 cc. +	
	*	*	—	++++	L + = 0.15 cc.	
	—	*	*	+++	L + = 1 cc.	

TABLE 2—*Continued*

Concentration of special constituents in 1000 cc.	Basic media: Beef infusion	Basic media: 2 per cent peptone	Basic media: 1 per cent Liebig's extract	Character of growth	Potency of toxin	Remarks
Sodium asparaginate 1.5 grams	*	—	—	++++	M. L. D. = 0.05 cc.	Behavior same as with glutaminic acid hydrochloride
	—	*	—	++	M. L. D. = 1 cc. +	
	—	—	*	+	M. L. D. = 1 cc. +	
	*	*	—	++++	L + = 0.15 cc.	
	—	*	*	+++	L + = 1 cc.	
Creatin, 0.2 gram; creatinin, 0.15 gram	*	—	—	+++	M. L. D. = 1 cc. +	Scant growth with beef infusion base
	—	*	—	++	M. L. D. = 1 cc. +	
	—	—	*	+	M. L. D. = 1 cc. +	
	*	*	—	++++	L + = 0.15 cc.	
	—	*	*	+++	L + = 1 cc.	
Xanthin, 0.05 gram; hypoxanthin, 0.05 gram	*	—	—	+	M. L. D. = 1 cc. +	Beef infusion growth same as preceding
	—	*	—	++	M. L. D. = 1 cc. +	
	—	—	*	+	M. L. D. = 1 cc. +	
	*	*	—	++++	L + = 0.15 cc.	
	—	*	*	+++	L + = 1 cc.	
Glucose	*	—	—	+	M. L. D. = 1 cc. +	Beef infusion growth same as with creatin
	—	*	—	++	M. L. D. = 1 cc. +	
	—	—	*	+	M. L. D. = 1 cc. +	
	*	*	—	++++	L + = 0.25 cc.	
	—	*	*	+++	L + = 1.5 cc.	
Glucoseamine HCl, 2.00 grams	*	—	—	+	M. L. D. = 1 cc. +	Behavior same as with glucose
	—	*	—	++	M. L. D. = 1 cc. +	
	—	—	*	+	M. L. D. = 1 cc. +	
	*	*	—	++++	L + = 0.2 cc.	
	—	*	*	+++	L + = 1.5 cc.	
Control	*	—	—	+	M. L. D. = 1 cc. +	Behavior same as preceding
	—	*	—	++	M. L. D. = 1 cc. +	
	—	—	*	+	M. L. D. = 1 cc. +	
	*	*	—	++++	L + = 0.15 cc.	
	—	*	*	+++	L + = 1 cc.	
NaCl, 4 grams; K_2HPO_4, 3 grams $MgSO_4$, 0.4 grams; KNO_3, 0.2 grams	*	—	—	+	M. L. D. = 1 cc. +	Behavior same as preceding
	—	*	—	++	M. L. D. = 1 cc. +	
	—	—	*	+	M. L. D. = 1 cc. +	
	*	*	—	++++	L + = 0.15 cc.	
	—	*	*	+++	L + = 1 cc.	

Tyrosin, leucin, and histidindichloride, when added to beef infusion, each appear to yield the same results. They permit a moderate growth of the organism, but no toxin of consequence is elaborated. Comparison of the protocols given by the remainder of the special constituents investigated with those furnished by the control preparations shows no essential difference. These include the results obtained with creatin and creatinin, xanthin and hypoxanthin, the hydrochloride of glucosamine, glucose, the inorganic ingredients, sodium chloride, magnesium sulphate, potassium nitrate and dipotassium hydrogen phosphate. The growth of the bacillus and formation of toxin in the various "control" media have already been discussed.

DISCUSSION.

Notwithstanding the fact that the amino acids by themselves appear to be unable to support the metabolism of *Bact. diphtheriæ,* some of these protein degradation products, as has been shown in the foregoing, have a decided influence on growth and toxin production with this organism. Of the various amino acids investigated, cystin seems to be of special importance. Addition of this sulphur-containing body not only permits heavy typical growth of the diphtheria bacillus in a medium like beef infusion, which is merely capable of maintaining metabolism of *Bact. diphtheriæ*, but simultaneously the organism is now able to elaborate a potent toxin. That this toxin is a true diphtheria toxin is amply demonstrated by its ability to neutralize diphtheria antitoxin, giving an L + value for potency closely corresponding with the theoretical value derived from determination of the minimum lethal dose.

The fact that tryptophane in beef infusion allows a growth of *Bact. diphtheriæ* practically of the same intensity as does cystin, while the resultant toxin is of inferior potency, would suggest that the sulphur complex in the cystin molecule enters into toxin production. It seems likely that diphtheria toxin contains sulphur, and that where tryptophane and other non-sulphur-containing amino acids permit toxin elaboration, with beef infusion, the sulphur portion of the toxin molecule is derived from the infusion. Indol tests made after growth of the organism in tryptophane-containing media were found to be negative, indi-

cating that in the utilization of this compound by the diphtheria bacillus, there is no intermediate separation of the indol ring.

The very close relationship of asparaginic acid (amino succinic acid) to its next higher homologue, glutaminic acid (amino glutaric acid) appears to be closely paralleled by the similarity in the protocols furnished by both glutaminic acid hydrochloride and sodium asparaginate. The growth of the organism is of equal intensity in presence of either of these substances and a toxin of practically the same potency is elaborated in both cases.

It is worthy of note that glycocoll added to beef infusion with a reaction adjusted to the proper H ion concentration permits of heavy growth and toxin elaboration with *Bact. diphtheriæ*. Since glycocoll has no asymmetric carbon atom, it might be deduced that the major factor contributing to formation of toxin in this case need not necessarily be optically active. Furthermore, although equivalent results were obtained with both sodium asparaginate and glutaminic acid hydrochloride, the glutaminic acid is dextrorotatory while the asparaginic acid turns the plane of polarized light to the left. Seemingly, the direction of rotation is also of minor consequence.

The fact that it was found impossible to grow *Bact. diphtheriæ* satisfactorily and obtain a production of toxin except in the presence of beef infusion, even though the quantity of the latter was relatively very small, suggests a food hormone requirement for growth, and particularly for toxin production, by the organism. Davis (1917) has discussed the question of food accessory factors with reference to cultures of hemophilic bacilli and both Lloyd (1917) and Drew (1917) with the meningococcus. They all conclude that the presence of these factors is essential. Assuming that both peptone and Liebig's Extract of beef are deficient in vitamines, and that ordinary beef infusion has these factors present, this may partially explain the scant growth and absence of toxin which obtained in presence of both the peptone and the extract.

It is readily appreciated that the preceding experimentation has not directly included other important amino acids, particularly proline and lysine, although these have been comprehended in the hydrolysis media nos. 9 and 10 detailed in table 1. The experimental data obtained, however, warrant the belief that it is not possible to maintain metabolism of *Bact. diphtheriæ* in a

medium composed entirely of amino acids, even after addition of muscle extractives and mineral salts.* This lends strength to the theory that diphtheria toxin is an excretory product formed by the organism only when certain amino acids and accessory factors, the latter perhaps of a vitamine character, are present. It does not seem likely, as has been claimed by several, that the toxin is a synthetic product, built up directly by *Bact. diphtheriæ* from mineral salts and nitrogen compounds of the character of amino acids.

SUMMARY.

1. *Bact. diphtheriæ* could not be cultivated in synthetic media composed of amino acids and mineral salts adjusted to the optimum H ion concentration. Addition of the extractives creatin and creatinin and the purine bases, xanthin and hypoxanthin, was of no advantage.

2. Typical luxuriant growth of *Bact. diphtheriæ* was obtained in a mixture of 99.5 per cent synthetic medium and only 0.5 per cent of bouillon. Production of active toxin, however, required the presence of 10 per cent bouillon.

3. Cystin, when added to plain beef infusion, a culture medium, just capable of maintaining growth of *Bact. diphtheriæ,* not only permitted rapid and heavy vegetation, but a strong toxin (minimum lethal dose equals 0.0075 cc) was also elaborated. This appears to be a true toxin capable of neutralizing the corresponding diphtheria antitoxin.

Tryptophane, under the same conditions, allowed almost as heavy growth, but the resultant toxin (minimum lethal dose equals 0.01 cc) was weaker in potency. Glutaminic acid hydrochloride and sodium asparaginate gave parallel results. The growths were not as heavy as with cystin or tryptophane and, in both cases, the toxin produced had only one-fifth of the strength (minimum lethal dose equals 0.05 cc). Heavy typical growth was also obtained with glycocoll, but the toxin elaborated (minimum lethal dose equals 0.07 cc) was much less potent.

Moderate growth and practically no toxin production by *Bact. diphtheriæ* were obtained in beef infusion containing leucin, tyrosin, or histidindichloride. Addition to beef infusion

*Experimentation in progress since this paper was read indicates that growth of *Bact. diphtheriae* in a 100 per cent synthetic medium is possible. This will be discussed in a later publication.

of any of the following: creatin and creatinin, xanthin and hypoxanthin, glucose, glucoseamine hydrochloride, and the inorganic salts, sodium chloride, dipotassium hydrogen phosphate, magnesium sulphate, and potassium nitrate, permitted only scant growth and consequently no toxin. Substitution of the beef infusion by either peptone or Liebig's Extract resulted in deficient growth and toxin formation. This and the data furnished by the synthetic media suggest a vitamin requirement not only for luxuriant growth of the organism but particularly for strong toxin production.

4. The results obtained favor the belief that diphtheria toxin is not a synthetic product, but rather a catabolic substance elaborated by *Bact. diphtheriæ* only in presence of certain amino acids and accessory factors, the latter probably of a vitamin character.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 209, 1919.

(Reprinted from *The Journal of Immunology*, Vol. IV, No. 3, May, 1919.)

A METHOD FOR THE PRODUCTION OF A HOMOGENEOUS SUSPENSION OF BACILLUS ANTHRACIS TO BE USED IN AGGLUTINATION REACTIONS.

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(From the Medical Research Laboratories of Parke, Davis & Company, Detroit, Mich.)

Because of the fact that an accurate and reliable method for the standardization of antianthrax serum has not yet been developed, while the necessity for such a method is very apparent, experiments were undertaken to make use of the agglutination and complement fixation reactions. So far the results obtained from the animal protection tests have not proved uniform, and therefore not satisfactory, mainly because the animals on which the serum is being tested are so highly susceptible to the disease, and because of the difficulty in standardizing the test culture and maintaining a standard dose, due to variations in the virulence of different strains of *B. anthracis*.

Of the serum reactions, the complement fixation test has thus far not proven to be of value, as a stable antigen has not been produced, though further experiments are being made along this line. With regard to agglutination, the results as reported in the literature appear quite conflicting. Sobernheim in reviewing the subject states that the agglutinating action of serum on anthrax bacilli may be observed both microscopically and macroscopically, though the immobility of the bacilli and their inclination to arrange themselves in clumps make the judgment of agglutination difficult; and that with many sera one always obtains distinct agglutination in strong dilution, while, on the

other hand, it is often lacking in even high grade anthrax serum. He gives the statements of various writers, briefly, as follows: Sawtschenko found that horse serum agglutinated in every case, irrespective of whether it came from normal or preventatively inoculated animals, while dog serum of both categories never evidenced agglutinating power. Contrary to the report made by Sobernheim, that the specifically agglutinating power is usually lacking in the anthrax serum, Cavine states that a series of various anthrax sera proved active, in dilutions of 1-50,000 to 1-150,000, or even 1-500,000. Gottstein obtained completely negative results in a retest of these experiments, several high grade sera from horses, cattle and sheep showing no agglutination power. Sobernheim states that the remarkable fact can be determined that a serum agglutinates, for example, the bacilli of virulent anthrax and of Pasteur's Vaccine I, but not of Vaccine II, while another perhaps has no influence on the virulent anthrax and Vaccine II, but gives a distinct agglutination with cultures of Vaccine I.

From these conflicting statements Sobernheim draws this conclusion:

"Although the question of agglutination for anthrax serum requires further explanation, we may say that a parallelism exists between the agglutinating and immunizing power of the serum in no case, and the presence or absence of agglutinating action has absolutely no connection with the degree of immunity in the serum producing animal."

It seems to the writer that the problem is largely, if not entirely, a problem of the suspension. Because of the nature of the organism, growing as it does in long chains and producing spores, it does not easily lend itself to agglutination experiments. However, a homogeneous suspension of *B. anthracis* has been prepared and an increase of agglutinins demonstrated in sera from horses treated with vaccines of *B. anthracis*, as against sera from normal horses.

The suspension for the agglutination tests was prepared as follows: The cultures employed were four strains of *B. anthracis* furnished by the United States Bureau of Animal Industry, and designated by them "Davis," "Chestertown," "N. H.," and "6071."

These four strains were transplanted daily for ten days on plain agar and incubated at 42.5° C., until a sporeless and very vigorous growth was obtained. Each strain was then planted on plain agar in quart whiskey flasks and incubated for twelve hours at 42.5°C. The growths were washed off in physiologic salt solution containing 0.5 per cent formalin (about 100 cc to a flask). The suspensions were shaken in a mechanical shaker for forty-eight hours. After standing for several days and being tested for sterility, equal parts of each suspension were mixed in a cylinder; shaken for twenty-four hours; and allowed to stand over night. The larger clumps settle out, leaving a homogeneous suspension above. This upper portion was poured off and filtered several times through four thicknesses of sterile cheese-cloth. The suspension was then diluted with physiologic salt solution plus 0.5 per cent formalin to a density corresponding to a suspension of *B. typhosus* containing 2000 million bacteria per cubic centimeter. A suspension of *B. anthracis* so prepared is perfectly homogeneous, stands up for at least forty-eight hours at 37° C. and shows no spontaneous agglutination.

The sera used were from thirteen horses which were treated first with vaccines of attenuated cultures and then with increasing doses of virulent *B. anthracis*. The strains were the same as used in the preparation of the suspensions. Also, sera from seven normal, untreated horses were tested.

The agglutination tests. All the agglutination tests were macroscopic. In carrying out the tests, the serum dilutions were made in test-tubes with physiologic salt solution. The dilutions were never started with less than 1 cc of undiluted serum, and the volume of each dilution was always more than sufficient for the test. Special pipettes, graduated to 0.5 and 1 cc, were employed throughout and a different pipette was used for each dilution. All glassware used in connection with the tests was clean and sterile. In the test, each small agglutination tube contained 0.5 cc of suspension plus 0.5 cc of diluted serum, with a control tube containing 0.5 cc suspension plus 0.5 cc salt solution. The tests were incubated at 37°C. for twenty-four hours.

The results of the agglutination reactions with antianthrax sera and normal horse sera are given in tables 1 and 2.

TABLE 1

SERUM DILUTION	RESULTS, JANUARY 17, 1919						
	Suspension — B. anthracis Sera —						
	Normal horse 901	Antianthrax, Horse					
		1057	1051	1050	1049	1047	1045
1-10	+++	+++	+++	+++	+++	+++	+++
1-20	+++	+++	+++	+++	+++	+++	+++
1-40	+++	+++	+++	+++	+++	+++	+++
1-80	++	+++	+++	+++	+++	+++	+++
1-200	+	+++	+++	+++	+++	+++	+++
1-400	–	+++	+++	+++	+++	+++	+++
1-800	–	+++	+++	+++	+++	+++	+++
1-1600	–	+++	+++	+++	+++	+++	+++
1-2000	–	+++	+++	+++	+++	+++	+++
1-3200		+++	+++	+++	++	++	+++
1-6400		+++	++	++	+	++	+
1-10000		++	+	–	+	+	+
1-20000		+	–	–	–	+	–
1-40000		–	–	–	–	–	–
Control	–	–	–	–	–	–	–

+++ represents complete agglutination; ++ partial; + slight agglutination, but still with positive clumping.

Each antiserum has been tested more than once and different bleedings from the same horse have been tested with practically no variation in the agglutination titer.

The sera from five normal horses, in addition to the two given in the tables, gave agglutination titers of from 1 in 80 to 1 in 200.

SUMMARY.

A satisfactory suspension of *B. anthracis*, for agglutination reactions, has been prepared by the described method. In order to be assured of a homogeneous suspension certain points must be observed. The cultures must be sporeless and must contain vigorous growths free from old organisms. The growths for the suspensions must be young, not more than eighteen hours old. The suspension must be thoroughly shaken; the larger clumps allowed to settle; and then carefully strained.

Agglutinins have been demonstrated in the serum from horses hyper-immunized with *B. anthracis*. The antianthrax sera from

TABLE 2

SERUM DILUTION	RESULTS, JANUARY 18, 1919							
	Suspension — B. anthracis Sera —							
	Normal horse 1665	Antianthrax, Horse						
		1042	1027	1025	957	956	955	953
1-10	+++	+++	+++	+++	+++	+++	+++	+++
1-20	+++	+++	+++	+++	+++	+++	+++	+++
1-40	++	+++	+++	+++	+++	+++	+++	+++
1-80	+	+++	+++	+++	+++	+++	+++	+++
1-200	−	+++	+++	+++	+++	+++	+++	+++
1-400	−	+++	+++	+++	+++	+++	+++	+++
1-800	−	+++	+++	+++	+++	+++	+++	+++
1-1600	−	+++	+++	+++	+++	+++	+++	+++
1-2000	−	+++	+++	+++	+++	+++	+++	+++
1-3200		+++	+++	++	+++	+++	+++	+++
1-6400		++	++	++	++	+	++	+++
1-10000		+	+	+	+	−	++	++
1-20000		−	−	−	−	−	+	+
1-40000		−	−	−	−	−	−	−
Control	−	−	−	−	−	−	−	−

thirteen horses have given agglutination titers of from 1 in 6400 to 1 in 20,000, as against titers of from 1 in 80 to 1 in 200 in normal horses.

The agglutination tests show that certain antibodies have been produced in horses treated with *B. anthracis*, and in the absence of a satisfactory animal protection test or method of complement fixation the agglutination test may be used as a method for standardizing antianthrax serum.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 210, 1919.

(Reprinted from *The Journal of Biological Chemistry*, Vol. XXXVIII, No. 2, 1919.)

THE STABILITY OF LACTALBUMIN TOWARD HEAT.

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(From the Medical Research Laboratories of Parke, Davis & Company, Detroit, Mich.)

Since there seems to be some specific and characteristic difference between the growth-promoting value of casein and lactalbumin, which is dependent upon certain dietary conditions as shown by comparing the results of Osborne and Mendel,[1] McCollum, Simmonds, and Parsons,[2] and Emmett and Luros,[3] we have made a further study of this subject by undertaking to ascertain whether heat will affect the nutritive value of lactalbumin to the extent that McCollum and Davis claim that it destroys the growth-promoting value of casein.

McCollum and Davis[4] reported that, by long continued heating of milk at 90-100°, the nutritive value of the casein was impaired; that, when casein was heated in an autoclave for 1 hour at 15 pounds pressure, it lost its "biological value as a complete protein," due to some of the amino-acids being broken down; that wet milk powder heated for a time in a double boiler or for 1 hour in the autoclave at 15 pounds pressure was deficient in promoting growth; that evaporated milk whey was not affected by heating in the autoclave; and that unrefined lactose retained as much of the adhering water-soluble B accessory after heating as before. In view of these findings, McCollum and Davis concluded that it would not be practicable to purify casein by washing it with hot alcohol as Funk and Macallum[5] had done, and so proposed a method of purification which did away with the use of heat entirely. Funk and Macallum in turn prepared casein by these two methods and found that there was no difference between them in respect to their growth-promoting value. Upon heating the casein in the autoclave, however, they obtained evidence which confirmed McCollum and Davis as to a loss in the biological value of casein. They attributed the change in the nutritive value of the protein to a destruction of the antiscorbutic vitamin,

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[1]Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1915, xx, 351; *ibid.*, 1916, xxvi, 1.

[2]McCollum, E. V., Simmonds, N., and Parsons, H. T., *J. Biol. Chem.*, 1919, xxxvii, 287.

[3]Emmett, A. D., and Luros, G. O., *J. Biol. Chem.*, 1919, xxxviii, 147.

[4]McCollum, E. V., and Davis, M., *J. Biol Chem.*, 1915, xxiii, 231, 247.

[5]Funk, C., and Macallum, A. B., *Z. physiol. Chem.*, 1914, xcii, 17; *J. Biol. Chem.*, 1916, xxvii, 51.

and not to a breaking down of the protein. Hogan[6] studied the effect of heat on the growth-promoting value of corn, egg albumen, and casein. His data indicate that when corn was heated in the autoclave, no growth resulted, while excellent results were obtained with unheated corn. Egg albumen, heated in the autoclave, and then combined with protein-free milk, butter, starch, and agar so as to make a 9 per cent protein diet, was very slightly, if at all, impaired. Upon heating casein in an autoclave under 15, 30, and 45 pounds pressure, respectively, for 2 hours, and making up three corresponding rations with protein-free milk, butter fat, starch, and agar, and comparing each with a ration containing unheated casein, Hogan obtained results which indicated that casein was not affected by heat. His conclusions were that heat does not lower the nutritive value of the casein, but that one or more of the food accessories may be affected.

In the series of tests reported here on the effect of heat upon the nutritive value of lactalbumin, we subjected this protein to different temperatures and then incorporated it in the basal diet. The control supply of lactalbumin was dried *in vacuo* at 55-60° (Chart 1). Portions of this lot were then heated as follows: (*a*) in the air oven at 90-100° for 16 to 18 hours (Chart 2); (*b*) in the autoclave at 15 pounds pressure for 2 hours (Charts 3, 4, and 5); and (*c*) in the autoclave at 15 pounds pressure for 6 hours (Chart 6). The basal diet was composed of protein-free milk 28 per cent; butter fat 5, 18, or 28 per cent; lard none or 10 per cent; and starch to make up the balance after adding the protein. In each case, 10 per cent of lactalbumin protein was employed, this being based upon the nitrogen content of each of the proteins.

The protocols accompanying the charts give the details of the different tests. It will be seen, from the curves showing the rate of growth for the different groups of rats, that heating the lactalbumin had no effect upon its growth-promoting value.

In view of this fact, it is of interest to compare these results with our former conclusion, that lactalbumin is a complete protein for growth,[3] and to correlate the evidence that we put forth to show that the difference between lactalbumin and casein may be based on the vitamin hypothesis.

Studying the basal diets of McCollum and Davis, Funk and Macallum, and Hogan, it will be seen that their experimental rations differed in two essential points: first, in respect to the amount of butter fat used, and second, in respect to the source of water-soluble vitamins employed. Thus, McCollum and Davis used 5 per cent butter fat and obtained their water-soluble vita-

[6]Hogan, A. G., *J. Biol. Chem.*, 1916, xxvii, 193; *ibid.*, 1917, xxx, 115.

mins from powdered milk, milk whey, wheat germ, or lactose. Funk and Macallum employed no butter fat but obtained the fat-soluble A accessory from dried yeast which also furnished the water-soluble B. Orange juice was also used. Hogan incorporated 30 per cent of butter fat in his rations, and for the water-soluble vitamins he used either corn, or protein-free milk.

From the standpoint of the amount of butter fat, it might be said that Hogan obtained normal growth with heated casein because he used 30 per cent of butter fat, while McCollum and Davis secured poor results on account of having only 5 per cent of the fat present. It is easy to conceive that the larger amount of butter fat might have altered the palatability of the ration so that the food intake was modified and thereby produced a difference in gain in weight; or it might be that this diet was more easily assimilated. Osborne and Mendel[7] and Maignon[8] found that the availability of protein varied directly with the amount of fat present. The data given in Charts 3, 4, and 5, where 18, 5, and 28 per cent, respectively, of butter fat was mixed with the 2 hour autoclave lactalbumin, indicate clearly that the amount of butter fat had no effect upon the rate of growth. One group of rats grew as well as another and the body condition of each was equally good. This is brought out more clearly perhaps when the food intake is considered. Table I shows that the group consuming the low fat diet ate more food than did the one on the high fat ration. In other words, the low fat group consumed much more protein, and if the heated lactalbumin had been toxic, we would

TABLE I.

Average Values for Each Group for Five Weeks.

Lactalbumin.	Fat.			Gain per day.	Food intake per day.	Gain per gm. of food.
	Butter fat.	Lard.	Total.			
	per cent	*per cent*	*per cent*	*gm.*	*gm.*	*per cent*
Unheated............	18	10	28	1.56	6.6	23.6
6 hour autoclave.....	18	10	28	1.50	7.8	19.2
2 " "	18	10	28	1.34	6.5	20.9
2 " " ...	28	—	28	1.72	7.7	22.8
2 " "	5	—	5	1.60	12.9	12.4

[7]Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1918, xxxiv, 521.
[8]Maignon, F., *Compt. rend. Acad.*, 1918, clxvii, 172.

not have obtained results which were equal to those made by the high fat group where less protein was ingested. Further, in comparing the food intake for the heated and unheated lactalbumin rations with 18 per cent butter fat and 10 per cent lard, the differences were so slight that it is evident that the heated protein was not toxic. These findings suggest that the percentage of butter fat in the ration had no special function in making the heated lactalbumin more available than the unheated protein, and it is more than likely that it had no influence upon the heated casein which Hogan used.

With regard to the water-soluble vitamins, in the case of McCollum and Davis' report, it so happened that in their various rations which contained milk or its components, they had present, when they obtained good growth, some constituents of the milk which had *not* been heated. Thus, with the heated casein they had unheated milk whey or lactose, while with the unheated casein they used heated whey or lactose. In other words, their combination of milk food substances was such that there was a good possibility that the unheated constituent carried an accessory (which we have already suggested as being able to be absorbed by lactose and possibly by casein) and that this was responsible for the growth on the rations. To be sure, the authors cover this point in part by showing that the wheat embryo can be heated for 1 hour in the autoclave at 15 pounds pressure and then when it is added to a diet of polished rice and butter fat, it is still able to supply the water-soluble B vitamin. However, from some of our results which are now in preparation for publication, we have evidence to show that heat will partially destroy a particular vitamin (other than the water-soluble B) that relates to growth, and that the rate of growth will depend largely upon the amount of the substance present and also upon the degree of heating. That is, if the minimum amount or slightly more of the unheated material, which carries the vitamin, is present, normal growth will occur, but heating this vitamin will cause poor growth or no growth at all. If, however, there is an excess of the vitamin present in the unheated food, the effect of heating it will not be so marked.

Funk and Macallum obtained poor growth with heated casein even when dried yeast was in the ration, but when 1 cc of orange

juice was added each day they obtained good results. This stimulation of growth was attributed to the antiscorbutic vitamin in the juice. Hogan supplemented each of his casein rations with protein-free milk just as we did with lactalbumin. Protein-free milk contains 80 per cent of lactose, and we[3] have shown that lactose has some peculiar biological property of stimulating growth which is due either to its ability to overcome toxicity or else to a vitamin (other than the water-soluble B) which it appears to absorb. Therefore, it may be that the lactose in the protein-free milk carried enough of the accessory or vitamin to effect normal growth with casein in Hogan's experiments. Chart 7 illustrates how the addition of lactose, as a partial substitute for starch in an otherwise complete diet, stimulated growth.

Our inference would be, therefore, that this accessory or vitamin was the factor that brought about the difference between the values of heated casein of McCollum and Davis on the one hand, and of Hogan on the other. This vitamin is different from the so called water-soluble B, which is stable toward heat. Whether it is the same as the antiscorbutic vitamin, as suggested by Funk and Macallum, is being studied. Pitz[9] claims that lactose used as a supplement to certain foods will prevent the onset of scurvy for 20 weeks. Cohen and Mendel[10] found that with very highly purified lactose scurvy was not retarded. In some preliminary experiments, we have found that our purified lactose was not efficacious in curing scurvy in guinea pigs in the advanced stage. Further study on the specific function of lactose is under way.

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[9]Pitz, W., *J. Biol. Chem.*, 1918, xxxiii, 471.

[10]Cohen, B., and Mendel, L. B., *J. Biol. Chem.*, 1918, xxxv, 425.

CONCLUSIONS.

From the results of the effect of heat on lactalbumin the data indicate: (1) that high temperatures do not influence the growth-promoting value of lactalbumin when dried *in vacuo* at 55°, or heated in an air oven at 90-100° for 16 hours, or in an autoclave at 15 pounds pressure for 2 and 6 hours, respectively; (2) that the amount of butter fat, whether 5, 18, or 28 per cent, when used with a 2 hour autoclaved lactalbumin, has no influence on the rate of growth; (3) that heated lactalbumin is not toxic for young growing rats; (4) that our previous conclusion regarding the excellent growth-promoting value of lactalbumin is further substantiated on the hypothesis that there is a vitamin factor involved which is different from the water-soluble B.

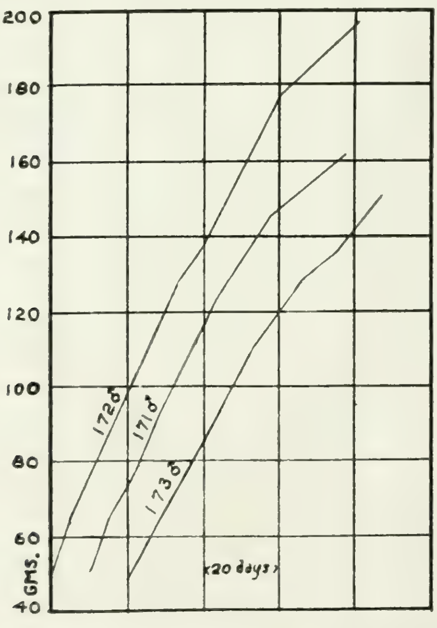


CHART 1. The rats in this group were fed a ration with 10 per cent lard and 18 per cent butter fat, and 10 per cent of lactalbumin protein which had been thoroughly purified by washing with hot water, then digesting a number of times with hot alcohol, and finally washing with ether. This lactalbumin was then dried *in vacuo* at 55-60° for 12 hours. Normal growth resulted.

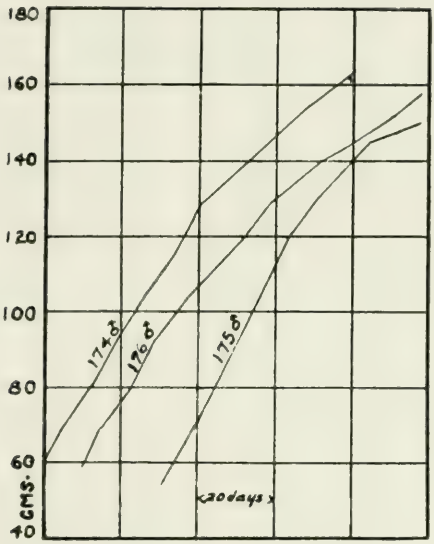


CHART 2. The diet for these rats was the same as that for the animals in Chart 1, except that the lactalbumin was heated at 90-100° for 16 to 18 hours in an air oven. There was very little difference in the rate of growth between this group and that fed the unheated lactalbumin.

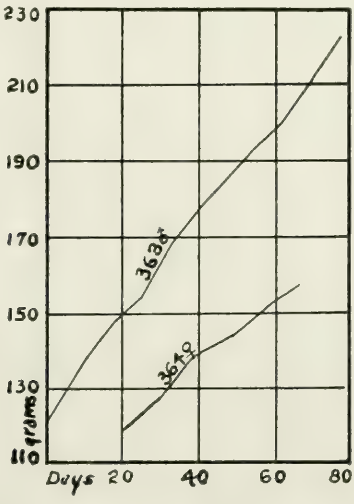


CHART 3. The diet differed from the control ration (Chart 1) in having 10 per cent of protein from lactalbumin which had been heated in the autoclave for 2 hours at 15 pounds pressure.

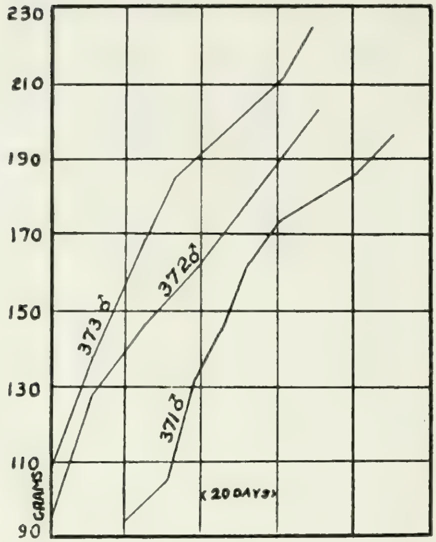


CHART 4. The ration was the same as that fed to the rats for Chart 3, except that only 5 per cent of butter fat and no lard were used.

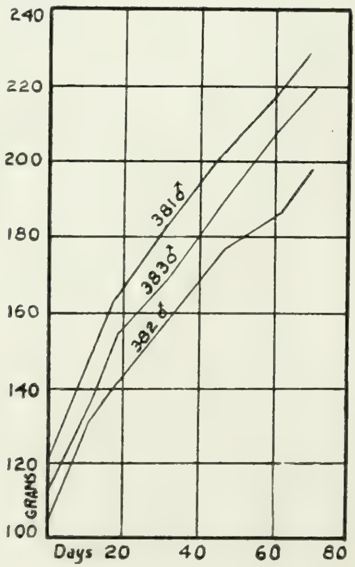


CHART 5. The ration used for these rats was the same as that fed to the group represented in Charts 3 and 4, except for the increase in butter fat to 28 per cent.

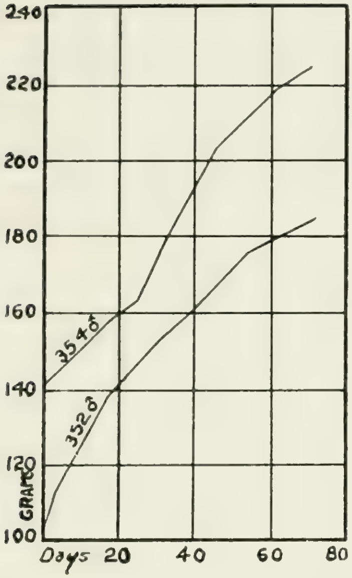


CHART 6. The ration differed from the control diet (Chart 1) and those for Charts 2 and 3, in having 10 per cent protein from lactalbumin which had been heated in the autoclave for 6 hours at 15 pounds pressure.

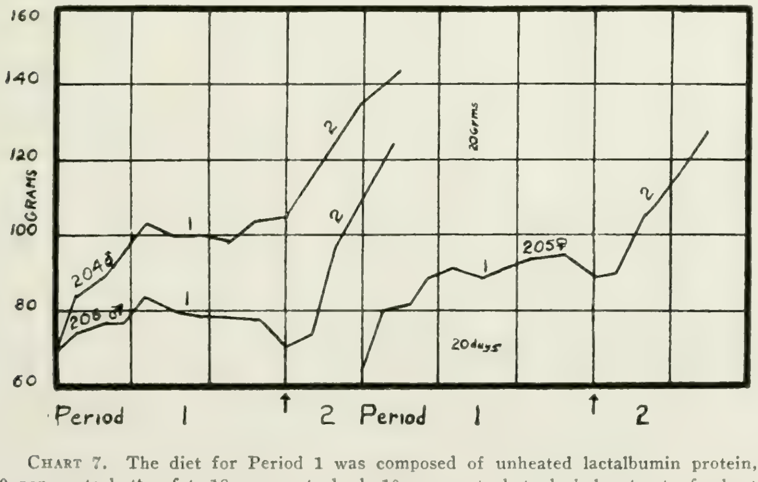


CHART 7. The diet for Period 1 was composed of unheated lactalbumin protein, 10 per cent; butter fat, 18 per cent; lard, 10 per cent; hot alcohol extract of wheat germ, 5 per cent; and starch to make up the balance. In Period 2, part of the starch was replaced by 24.6 per cent of purified lactose; otherwise the diet was the same as in Period 1. This shows that the addition of lactose stimulated growth.

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 211, 1919

(Reprinted from *The Journal of Biological Chemistry*, Vol. XXXVIII, No. 2, 1919.)

NUTRITIONAL STUDIES ON THE GROWTH OF FROG LARVÆ (RANA PIPIENS).

FIRST PAPER.

By A. D. Emmett and Floyd P. Allen.

With the Cooperation of G. O. Luros and M. Sturtevant.

(From the Medical Research Laboratories of Parke, Davis & Company, Detroit, Mich.)

Plates 2 to 5.

A number of studies have been reported upon the growth and development of frog larvæ as influenced by feeding certain products *in toto,* such as glandular materials. As far as we are aware, however, no one has incorporated in the feeding of such larvæ conditions which were intended to control definitely the nutritive value of the diet in terms of protein, carbohydrate, fat, mineral salts, and the food accessories, hormones or vitamins. We have, therefore, attempted in this preliminary report to give a summary of our studies along this line with a view of supplementing it from time to time in the light of further findings.

Hopkins, Osborne and Mendel, McCollum and associates, and several others have made, during the last few years, valuable contributions, in their studies with rats and mice, to the nutritive value of synthetic food mixtures. They have shown that proteins, fats, salt mixtures, and vitamins each occupy a peculiar place in the physiological economy—not alone in respect to the amount present in diet but also in respect to the quality or kind of each nutrient.

It was thought by using this particular species of amphibians, namely frog larvæ (*Rana pipiens*) which have a characteristic life cycle—passing through the tadpole stage to the frog—that we might be able to determine some interesting facts regarding their nutrition with respect to size and degree of metamorphosis. One great advantage in using such larvæ in place of larger animals

is that one can work with large numbers and thereby be able to draw fairly definite conclusions. On the other hand, it is readily seen that serious objections may be raised in the making of practical tests. For example, if the larvæ are fed in large groups there is no way of measuring the food intake, or of preventing the more vigorous ones from taking the food from the weak, or of eating the tadpoles that die occasionally, and no means of controlling the water-soluble content of the diet if water is allowed to flow through the trays continuously.

EXPERIMENTAL.

The tadpole eggs were collected in the near vicinity and brought to the laboratory with every precaution to prevent injury, since in the late stages of segmentation the eggs are very delicate. Immediately after hatching, the tadpoles were grouped into colonies of 500 each, being placed in enamel-lined trays. Special attention was paid to the hygienic conditions of the surroundings. Thus, the ventilation of the room, which was used only for this work, was regulated; the bright light was eliminated; the trays were thoroughly cleaned at regular intervals; unused food was removed frequently to prevent the formation of toxic substances due to decomposition; and fresh water was supplied two to three times daily. The water used was from the city supply, but it was first passed through a Hygeia filter which removed the suspended organic matter.

In order to obtain a permanent record of the progress of growth, photographs were taken each week of a selected group from each colony. The selection was governed by choosing from each tray an equal number of the largest, medium, and smallest specimens. Shadow photographs were made by subjecting the tadpoles to an instantaneous exposure of an arc light. From these photographic records, measurements were made of all the tadpoles, including body width, body length, and total length with tail.

Late in the experiment when the limb buds began to appear, microscopic examination of all the tadpoles in each tray was made. Following a definite scheme, each tadpole was classified according to its stage of development, and from these data the

percentage distribution of the tadpoles was calculated for the groups that were on different diets. In addition to this an attempt was made to preserve each week representative specimens from every group and to make a histological study of the serial sections made from these specimens. The individual variations between the tadpoles proved to be too great a factor, and for this reason the histological data will not be reported at this time. Text-fig. 1, page 177, illustrates the scheme followed in determining the various stages of development of the hind legs of the frog larvæ.

The feeding of the colonies or groups was done at specified times, always following the cleaning of the trays. The endeavor was to feed enough yet not too much, so as to avoid an excess of material remaining in the trays. This was governed in part by weighing out the food.

The synthetic diets were made up of various combinations of food substances: for protein, lactalbumin (1) and corn gluten (2) were used; cystine was also introduced as a supplement to corn gluten; for carbohydrate, purified starch or dextrin was used, and lactose, where Osborne and Mendel's protein-free milk (3) was incorporated; for fat, butter fat was employed as a carrier of fat-soluble A, lard was also used; for mineral salts, the McCollum Salt Mixture 185 was taken (4) except when protein-free milk was in the diet; for water-soluble B vitamin, either an alcohol extract of dried brewers' yeast, or protein-free milk was selected; and for another source of fat-soluble A, other than butter fat, we took an alcohol extract of linseed oil meal (5) which had first been treated with ether to remove the oil. Table I gives the composition of the diets fed.

DISCUSSION.

I. *Effect of Diet on the Size of the Tadpole.*

(*A*) *Influence of the Amount of Fat.*—In Table I, it will be seen that the rations or diets were arranged in two general classes, Groups I and II. The former was made up in accordance with the usual formula proposed by Osborne and Mendel; that is, these diets were high in fat, containing from 23 to 28 per cent of either lard alone or a mixture of lard and butter fat. In group II, the McCollum formula was followed as to fat; *i. e.,* 5 per cent.

TABLE I.

*Percentage Composition of the Diets.**

Diet No.	Foods in diet.	per cent	Diet No.	Foods in diet.	per cent
I_1	Lactalbumin (protein).	10.0	II_1	Lactalbumin (protein).	10.0
	Protein-free milk, purified.	28.0		Salt mixture.†	3.7
	Butter fat.	18.0		Butter fat.	5.0
	Lard.	10.0		Yeast extract (alcoholic.)	1.0
I_2	Lactalbumin (protein).	10.0	II_2	Lactalbumin (protein).	10.0
	Salt mixture.†	3.7		Salt mixture.†	3.7
	Butter fat.	18.0		Butter fat.	5.0
	Lard.	10.0			
I_3	Lactalbumin (protein).	10.0	II_3	Lactalbumin (protein).	10.0
	Protein-free milk, purified.	28.0		Salt mixture.†	3.7
	Lard.	28.0		Yeast extract (alcoholic).	1.0
I_4	Lactalbumin (protein).	10.0	II_4	Lactalbumin (protein).	10.0
	Salt mixture.†	3.7		Salt mixture.†	3.7
	Lard.	28.0		Lard.	5.0
I_7	Lactalbumin (protein).	10.0	II_7	Lactalbumin (protein).	10.0
	Protein-free milk, purified.	28.0		Salt mixture.†	3.7
	Linseed meal extract (alcoholic).	5.0		Linseed meal extract (alcoholic).	5.0
	Lard.	23.0		Yeast extract (alcoholic).	1.0
				Lard.	5.0
I_9	Lactalbumin (protein).	10.0	II_9	Lactalbumin (protein).	10.0
	Protein-free milk, purified.	28.0		Salt mixture.†	3.7
	Yeast extract (alcoholic).	1.0		Yeast extract (alcoholic).	1.0
	Lard.	27.0		Lard.	5.0
III	Corn gluten (protein).	10.0	III_4	Corn gluten (protein).	10.0
	Salt mixture.†	3.7		Cystine.	0.2
	Butter fat.	5.0		Salt mixture.†	3.7
	Yeast extract (alcoholic).	1.0		Butter fat.	5.0
				Yeast extract (alcoholic).	1.0

TABLE I.—*Concluded.*

Diet No.	Foods in diet.		Diet No.	Foods in diet.	
		per cent			*per cent*
II_{1a}	Lactalbumin (protein).	30.0	II_{2a}	Lactalbumin (protein).	30.0
	Salt mixture.†	3.7		Salt mixture.†	3.7
	Butter fat.	5.0		Butter fat.	5.0
	Yeast extract (alcoholic).	1.0			
IV_4	Lactalbumin (protein).	15.0	IV_5	Lactalbumin (protein).	15.0
	Salt mixture.†	3.7		Salt mixture.†	3.7
	Butter fat.	5.0		Butter fat.	5.0
	Yeast extract (alcoholic).	1.0			
IV_6	Lactalbumin (protein).	15.0	IV_8	Lactalbumin (protein).	15.0
	Salt mixture.†	3.7		Salt mixture.†	3.7
	Lard.	5.0		Yeast extract (alcoholic).	1.0
IV_{10}	Beef tissue (protein).	15.8	V_1	Lactalbumin (protein).	5.0
	Oats, rolled (protein).	1.9		Corn gluten (protein).	5.0
	Salt mixture.†	3.7		Salt mixture.†	3.7
	Butter fat.	5.0		Butter fat.	5.0
	Yeast extract (alcoholic).	1.0		Yeast extract (alcoholic).	1.0
III_6	Lactalbumin (protein).	10.0	VI_2	Lactalbumin (protein).	30.0
	Salt mixture.†	3.7		Salt mixture.†	3.7
	Butter fat.	5.0		Lard.	5.0
	Yeast extract (alcoholic).	1.0			

* In these rations purified starch was used in making up the total percentage, except in Ration III_6 where dextrin was employed.

† McCollum, E. V., Simmonds, N., and Pitz, W., *J. Biol. Chem.*, 1916, xxv, 105. Salt Mixture 185.

It will be seen from Fig. 1 that the tadpoles fed the high fat diet (I_1) were smaller than those that were fed the low fat diet (II_1). The periods referred to were 1 week apart. These permanent records were given from the time that differences began to be evident. The diets in these two cases were complete in the sense that they were sufficiently well balanced as to all the essential nutrients needed to produce normal growth in young rats.

(*B*) *Influence of the Vitamins.*—Inasmuch as the low fat diet appeared to be best for the tadpole, it is obvious that any comparisons between groups with respect to their nutritive value should be made on this plane. If the diets from Groups II_1 and II_2 are compared, it will be seen that they differed in that the yeast vitamin was removed from the former and an equivalent amount of starch substituted for it. In Fig. 2, the control Group II_1 showed distinctly larger tadpoles than Group II_2, up to Period 10, when the differences were not nearly so marked. Whether this convergence was due to the fact that the diet for Group II_1 may have lacked some essential constituent which retarded a more rapid growth should be borne in mind. It is evident that the water-soluble B was essential up to a certain stage in the growth. Beyond this it may be that the tadpole was able to store enough of this form of vitamin in the tail to supply its needs, just as certain bacteria appear to need vitamins at the start but later they synthesize the vitamins themselves (6).

Ration II_3 was deficient in fat-soluble A vitamins, but in other respects it corresponded to Ration II_1, lard being substituted for butter fat. From Fig. 3 it is evident that the tadpoles fed Ration II_1 grew better than those that lacked the fat-soluble A. After the eleventh period, however, the differences were too slight to be significant. In other words, the tadpoles seemed to need the fat-soluble accessory more in the early stages of growth, while later on there was an adaption, or a reserve supply, furnished through the medium of the tail.

When both vitamins were withdrawn from the diet, the tadpoles did not grow so vigorously. Contrary to what we have found with rats, however, they did show a gradual rate of growth. This may have been due in part to their ability to draw on the reserve stored in the tail, for in each instance the tail length was much shorter than with the tadpoles fed Ration II_1. In all cases, though, the body length and body width were also less, so that there was a general reduction in body size. Figs. 4 and 5 illustrate this. In the former case, the diet was based on a 10 per cent protein plane and in the latter case on a 30 per cent protein plane. The differences were more marked on the higher protein diet (Fig. 5).

(*C*) *Effect of the Amount of Protein.*—Osborne and Mendel

(7) and Emmett and Luros (8) have been able to obtain normal growth with rats with 10 per cent of lactalbumin protein. Osborne and Mendel found that it required 50 per cent more casein and 90 per cent more edestin than lactalbumin to bring about normal growth in rats. Wheeler (9) in working with mice determined that the best results for growth were obtained when the casein was as high as 30 per cent. It might therefore be that while lactalbumin is one of the very best proteins for promoting growth, the quantity needed for tadpoles should be higher than for rats.

Fig. 6 shows that when the protein plane was 30 per cent (II_{1^a}) the tadpoles grew more rapidly than when it was 10 per cent (II_1). In the former case the body width, length, and total length were greater than for Group II_1. It should be stated that Group II_{1^a} was obtained by subdividing Group II_1 in Period 9.

(*D*) *Effect of the Kind of Carbohydrate.*—In the large majority of the low fat diets, starch which had been purified by washing with hot 95 per cent alcohol was used as the sole source of carbohydrate. McCollum used dextrin in many of his rations, and since the low fat diets were based essentially upon his formula, we incorporated dextrin in Ration III_6 to determine whether it was an essential factor and would accelerate growth. The comparative value of the two rations, II_1 and III_6—the former starch and the latter dextrin—is shown in Fig. 7. There was practically no difference in the size of the two groups of tadpoles.

(*E*) *Value of Beef and Oat Diet.*—It is known that tadpoles will thrive on a diet of beef and oats. We therefore made up a control diet which was the same as Diet II_1 with the exception that we used 15.8 per cent of desiccated ether-extracted beef tissue protein and 1.9 per cent of ether-extracted rolled oats protein. Fig. 8 shows that we obtained our optimum growth with this ration. That this result was not due to the large amount of protein is evident, for when Fig. 6, which shows tadpoles that were fed 30 per cent protein, is compared with Fig. 8, the beef-oats diet was the best although it contained 17.7 per cent protein.

To what this difference was due we are not as yet able to state. However, in comparing the two rations II_1 and IV_{10}, it is evident that the beef tissue and rolled oats were the contributing factors; and that the presence of the butter fat, yeast extract, starch, and

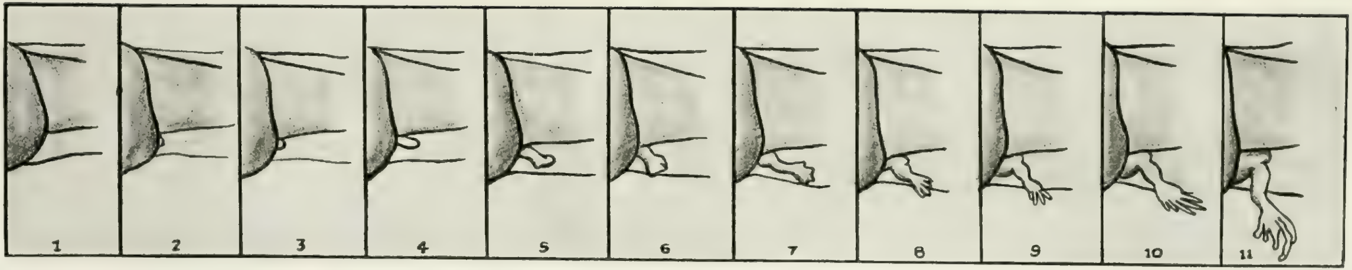
salt mixture in the diet did not inhibit growth. McCollum, Simmonds, and Parsons (10) claim that lactalbumin is an incomplete protein for growth. Emmett and Luros (8) found under certain conditions that they were not able to obtain good growth with lactalbumin, but that the apparent trouble lay in the fact that this protein was either susceptible to toxic substances or else it needed the presence of a water-soluble vitamin other than the water-soluble B. When lactose or the protein-free milk of Osborne and Mendel was present, normal growth was obtained. It would appear that perhaps a similar condition of affairs took place with the tadpoles. This question is now being investigated.

II. Effect of Diet on Development of Hind Legs of Tadpoles.

Growth and development may bear only a limited relation to each other in tadpoles maintained in an artificial environment. To state that a specimen of certain measurements is in a corresponding stage of metamorphosis may, upon closer inspection, prove untrue. Individual variation in both respects has been found by us to be marked. Consequently the colonies should be fairly large and observation should be made on as many members as possible in order that the true average may be most nearly approached. Bearing this in mind, all the tadpoles in each of the groups were examined as to the stage of development. This, as was previously stated, was not carried out in the observations made for growth or size.

(*A*) *Influence of the Amount of Fat in the Diet.*—In Table II are presented the data giving the percentage distribution of the tadpoles in each group with respect to the stage of development of the hind legs. These data, for two of the experimental periods (Nos. 12 and 14), near the close of the series of tests, are arranged chiefly with reference to the amount of fat in the diet, and also the kind of vitamin that is present. The detailed percentage composition of the diets is given in Table I. It is to be noted that the group number and diet number correspond. Thus, in Table II, Group I_1 was fed Diet I_1.

If Group I_1 (28 per cent fat) is compared with Group II_1 (5 per cent fat) it is seen, when both the water-soluble B and fat-soluble A accessory substances were present, that the tadpoles



TEXT-FIG. 1. The above drawings illustrate the scheme followed in determining the classification of the frog larvæ with respect to the development of the hind legs—as used in Tables II, III, and IV.

TABLE II.

Influence of Amount of Fat upon Development of Hind Legs of Rana pipiens Larvæ.

Percentage Distribution within Groups.

Group.	Diet.		Experimental period.	Stages of development of hind legs.*										
	Class of vitamines.	Fat.		1	2	3	4	5	6	7	8	9	10	11
		per cent												
I_1	Water- and	28†	12	11.1	62.9	22.9	6.3	—	—	—	—	—	—	—
I_1	fat-soluble.	28†	14	—	45.4	36.3	18.2	—	—	—	—	—	—	—
II_1	" " "	5‡	12	4.2	29.8	34.0	8.5	6.4	12.8	—	2.1	—	2.1	—
II_1	" " "	5‡	14	—	14.6	34.1	29.5	7.3	2.4	—	4.9	2.4	4.9	—
I_7	Water- and	23§	12	8.1	43.9	40.0	8.1	—	—	—	—	—	—	—
I_7	fat-soluble.	23§	14	6.6	50.4	32.4	10.4	—	—	—	—	—	—	—
II_7	" " "	5§	12	9.1	35.6	37.4	14.1	1.1	1.1	1.1	—	—	—	0.5
II_7	" " "	5§	14	8.6	16.8	47.0	21.6	3.6	—	1.2	1.2	—	—	—
I_2	Fat-soluble.	28†	12	31.3	43.4	20.2	5.1	—	—					
I_2	"	28†	14	28.3	35.8	25.0	10.9	—	—					
II_2	"	5‡	12	9.4	40.5	36.5	12.2	1.4	—					
II_2	"	5‡	14	—	20.3	53.0	17.2	7.8	1.6					
I_3	Water-sol-	28§	12	11.4	56.1	26.5	5.3	0.8	—	—	—	—	—	—
I_3	uble.	28§	14	13.7	34.4	41.0	10.7	—	—	—	—	—	—	—
II_3	"	None.	12	1.5	12.1	34.8	24.2	15.1	9.1	—	1.5	—	—	1.5
II_3	"	"	14	3.3	13.1	18.0	41.0	8.2	3.3	8.2	3.3	1.6	—	—
I_9	Water-sol-	27§	12	—	65.3	26.5	8.2	—						
I_9	uble.	27§	14	—	68.9	17.3	13.8	—						
II_9	"	5§	12	23.9	51.4	17.4	6.5	0.7						
II_9	"	5§	14	17.6	30.4	39.2	10.8	1.9						
I_4	None.	28§	12	11.2	46.5	33.6	8.6	—						
I_4	"	28§	14	10.2	35.1	32.4	21.3	0.9						
II_4	"	5§	12	17.0	56.2	19.5	7.3	—						
II_4	"	5§	14	16.6	39.0	28.0	16.6	—						

* See Text-fig. 1, p. 177.

† 18 per cent butter fat and 10 per cent lard.

‡ Butter fat.

§ Lard.

fed the low fat diet developed much better. In Groups I_7 and II_7, the fat-soluble A was supplied by an alcoholic extract of linseed meal, whereas it was furnished in the two preceding groups by butter fat. The difference between these two groups was clearly in favor of the low fat diet. With Groups II_1 and II_7, we have a comparison of two kinds of fat, butter fat and lard, and it appears that 5 per cent of either was permissible for fair development. Whether a smaller amount would have been much better is being tried out.

When the diet lacked the water-soluble B vitamin and contained the fat-soluble A in the form of butter fat, as shown in Groups I_2 and II_2, the low fat diet produced the best results. The differences were not so marked, however, as in the cases of Diets I_1, I_7, II_1, and II_7.

Groups I_3 and II_3 were fed diets which were deficient in fat-soluble A. In the case of the latter group no fat was used. It will be seen that this group was far in advance of the high fat-fed colony. In fact, there is such a marked contrast that the question immediately arises as to what extent Group II_1 would have developed if we had used some other source of fat-soluble A than a fat, as butter fat, and what results would have been obtained if the lard had been omitted from Diet II_7. In Groups I_9 and II_9, this point is partially answered since the diet for II_9 differed only from that of II_3 in having 5 per cent lard added to it and in having a corresponding decrease in starch. It would appear in comparing Groups II_9 and II_3 that the fat had a marked inhibiting effect. With respect to Groups I_9 and II_9, which contained 27 and 5 per cent of lard respectively, there was only a slight difference in favor of the lower fat diet.

When both vitamins were absent, as in the Groups I_4 and II_4, the high fat diet appeared to produce somewhat better results than the low fat diet.

It is evident from these data that the amount of fat in the diet had an appreciable influence upon the development of the tadpole, *i. e.,* the higher the fat content, the lower the stage of development.

(*B*) *Influence of Vitamins.*—In Table III, the data for the percentage distribution of the tadpoles within the groups are arranged with respect to the presence or absence of vitamins in

TABLE III.

Influence of Vitamines upon Development of Hind Legs in Rana pipiens **Larvæ.** *Percentage Distribution within Groups.*

Group.	Diet.		Experimental period.	Stages of development of hind legs.*										
	Class of vitamines.	Fat.		1	2	3	4	5	6	7	8	9	10	11
		per cent												
I_1	Water- and fat-soluble.	28†	12	11.1	62.9	22.9	6.3	—						
I_1	" " "	28†	14	—	45.4	36.4	18.2	—						
I_7	" " "	23‡	12	8.1	43.9	40.0	8.1	—						
I_7	" " "	23‡	14	6.7	50.5	32.4	10.4	—						
I_2	Fat-soluble.	28†	12	31.3	43.4	20.2	5.1	—						
I_2	"	28†	14	28.2	35.9	25.0	10.9	—						
I_3	Water-soluble.	28‡	12	11.4	56.1	26.5	5.3	0.8						
I_3	"	28‡	14	13.7	34.3	41.2	10.8	—						
I_9	"	28‡	12	—	65.3	26.5	8.2	—						
I_9	"	28‡	14	—	68.9	17.3	13.8	—						
I_4	None.	28‡	12	11.2	46.5	33.6	8.6	—						
I_4	"	28‡	14	10.2	35.2	32.4	21.3	0.9						
II_1	Water- and fat-soluble.	5§	12	4.2	29.8	34.0	8.5	6.4	12.8	—	2.1	—	2.1	—
II_1	" " "	5§	14	—	14.6	34.1	29.5	7.3	2.4	—	4.9	2.4	4.9	—
II_7	" " "	5‡	12	9.1	35.6	37.4	14.1	1.1	1.1	1.1	—	—	—	0.5
II_7	" " "	5‡	14	8.6	16.8	47.0	21.6	3.6	—	1.2	1.2	—	—	—
II_2	Fat-soluble.	5§	12	9.4	40.5	36.5	12.2	1.4	—	—	—	—	—	—
II_2	"	5§	14	—	20.3	53.1	17.2	7.8	1.6	—	—	—	—	—
II_3	Water-soluble.	None.	12	1.5	12.1	34.8	24.2	15.1	9.1	—	1.5	—	—	1.5
II_3	"	"	14	3.3	13.1	18.0	41.0	8.2	3.3	8.2	3.3	1.6	—	—
II_9	"	5‡	12	23.9	51.4	17.4	6.5	0.7	—	—	—	—	—	—
II_9	"	5‡	14	17.6	30.4	39.2	10.8	1.9	—	—	—	—	—	—
II_4	None.	5‡	12	17.0	56.2	19.5	7.3	—	—	—	—	—	—	—
II_4	"	5‡	14	16.6	39.0	27.8	16.6	—	—	—	—	—	—	—

* See Text-fig. 1, p. 177.

† 18 per cent butter fat and 10 per cent lard.

‡ Lard.

§ Butter fat.

the diet. The upper half of the table gives the results for the high fat diets. It would appear that the excess of fat was such a large factor that there was practically no difference between the groups.

In regard to the low fat diet Group II_1 was somewhat further advanced than Group II_7. This may have been due to the lard or to the fact that the fat-soluble A accessory was supplied either qualitatively or quantitatively to a less degree than in II_1. When Groups II_1, II_2, and II_9 are compared, the withdrawal of either the water-soluble B (II_2) or of the fat-soluble A (II_9) resulted in a distinct inhibition or retardation in the development of the tadpoles. In the case of Group II_3, two factors seemed to have played a part, the lack of both fat-soluble A and of any fat. As a result, this group developed at practically the same rate as Groups II_1 and II_7. This would tend to suggest that the water-soluble B vitamin was more essential than the fat-soluble A, or that the tadpoles had a reserve supply of this latter accessory stored in the tail, upon which they could draw. When both vitamins were removed from the diet, however, it appears from Group II_4 that there was little or no reserve vitamin supply available, for these tadpoles developed more slowly than any of the other groups.

The data indicate that both vitamins are essential to the development of the tadpole; that when fat was present to the extent of 5 per cent and the fat-soluble A was absent, there was a marked suppression, but if there was no fat present, the rate of development appeared to be normal when compared with the groups fed the control diets (II_1 and II_7). When the water-soluble B vitamin was absent the cessation of the development of the tadpoles was very noticeable.

(*C*) *Influence of the Amount and Kind of Protein.*—The data in Table IV are so arranged that the values can be compared with respect to the amount of protein. There is sufficient information given to indicate the vitamins and also the amount and kind of fat that were present. Groups II_1, IV_4, III_7, and II_7 were fed lactalbumin in amounts of 10, 15, 30, and 10 per cent respectively with both vitamins and 5 per cent of fat. The results indicate that the amount of protein had no influence on the rate of development. When the water-soluble B accessory was absent and the

TABLE IV.

Influence of Amount and Kind of Protein upon Development of Hind Legs of Rana pipiens Larvæ. Percentage Distribution within Groups.

Group.	Diet. Protein. Kind.		Class of vitamines	Fat.	Experimental period.	Stages of development of hind legs.* 1	2	3	4	5	6	7	8	9	10	11
		per cent		*per cent*												
II_1	Lactalbumin.	10	Water and fat soluble.	5†	12	4.2	29.8	34.0	8.5	6.4	12.8	—	2.1	—	2.1	—
II_1	"	10	" " "	5†	14	—	14.6	34.7	29.5	7 4	2.4	—	4.9	2.4	4.9	—
IV_4	"	15	" " "	5†	12	5 1	21.4	21 4	26.6	15.2	3.8	2.5	1.3	—	2.5	—
IV_4	"	15	" " "	5†	14	—	32.5	47 5	5.0	2.5	7.5	2.5	2.5	—	—	—
III_7	"	30	" " "	5†	12	2.8	13 9	36.1	22.2	13.9	—	—	11.1	—	—	—
III_7	"	30	" " "	5†	14	—	3.4	17.2	37 9	10.4	10.4	6.9	—	3.4	3.4	6.0
II_7	"	10	" " "	5‡	12	9.1	35.6	37.4	14.1	1.1	1.1	1.1	—	—	—	0.5
II_7	"	10	" " "	5‡	14	8.4	16.8	47.0	21.6	3.6	—	1.2	1.2	—	—	—
III_6§	"	10	" " "	5†	12	0.8	23.7	38.1	17.9	6.8	6.0	1.7	0.8	2.5	—	0.8
III_6§	"	10	" " "	5†	14	2.3	25.5	38.9	15.0	5.5	4.0	5.5	1.6	0.8	—	0.8
II_2	Lactalbumin.	10	Fat-soluble.	5†	12	9.4	40.5	36.5	12.2	1.4	—					
II_2	"	10	"	5†	14	—	20.3	53.1	17.2	7.8	1.6					
IV_5	"	15	"	5†	12	9.9	43.2	36.0	10.8	—	—					
IV_5	"	15	"	5†	14	3.8	22.6	47.2	22.8	—	1.9					
II_{2a}	"	30	"	5†	12	27.3	34.5	21.8	12.7	1.8	1.8					
II_{2a}	"	30	"	5†	14	36.0	12.0	18.0	20.6	6.0	2.0					

II_3	Lactalbumin.	10	Water-soluble.	None.	12	1.5	12.1	34.8	24.2	15.1	9.1	—	1.5	—	—	1.5
II_3	"	10	"	"	14	3.3	13.1	18.0	41.0	8.2	3.3	8.2	3.3	1.6	—	—
IV_8	"	15	"	"	12	3.1	34.6	22.3	23.8	13.1	0.8	2.3	—	—	—	—
IV_8	"	15	"	"	14	8.3	28.8	26.7	24.2	6.7	5.3	—	—	—	—	—
VI_1	"	30	"	"	12	3.0	19.9	28.8	38.0	7.6	3.0	—	—	—	—	—
VI_1	"	30	"	"	14	8.3	1.7	30.0	35.0	16.6	5.0	3.3	—	—	—	—
II_4	Lactalbumin.	10	None.	5‡	12	17.0	56.2	19.5	7.3	—						
II_4	"	10	"	5‡	14	16.6	39.0	27.8	16.6	—						
IV_6	"	15	"	5‡	12	5.1	50.0	31.6	12.5	0.7						
IV_6	"	15	"	5‡	14	10.3	20.6	41.1	16.9	1.8						
VI_2	"	30	"	5‡	12	11.9	45.2	23.8	19.0	—						
VI_2	"	30	"	5‡	14	23.6	20.6	38.2	15.0	2.9						
IV_{10}	Beef and oat.	17.7	Water- and fat-soluble.	5†	12	4.6	18.9	24.5	26.5	11.8	6.6	2.5	2.5	—	2.0	—
IV_{10}	" "	17.7	" " "	5†	14	4.3	16.9	21.8	22.5	14.1	7.6	4.9	0.5	2.1	2.1	3.2
II_1	Lactalbumin.	10	" " "	5†	12	4.2	29.8	34.0	8.5	6.4	12.8	—	2.1	—	2.1	—
II_1	"	10	" " "	5†	14	—	14.6	34.1	29.5	7.3	2.4	—	4.9	2.4	4.9	—
V_1	Lactalbumin, corn gluten. Ratio 1:1.	10	Water- and fat-soluble.	5†	14	2.4	23.8	42.8	21.4	4.8	1.2	2.4	—	1.2		
III_4	Corn gluten, cystine.	10	Water- and fat-soluble.	5†	12	12.2	49.0	30.0	2.8	2.8	3.0					
III_4	" " "	10	" " "	5†	14	10.5	41.1	32.6	10.5	3.2	2.1					
III_5	Corn gluten.	10	Water- and fat-soluble.	5†	12	13.6	41.9	40.7	3.7	—						
III_5	" "	10	" " "	5†	14	5.6	45.0	39.4	8.4	1.4						

* See Text-fig. 1, p. 177.

† Butter fat.

‡ Lard.

§ Same as II_1 except dextrin used for starch.

percentage of lactalbumin varied from 10 to 30 per cent (Groups II_2, IV_5, and II_{2^a}), the 30 per cent protein diet (II_{2^a}) was somewhat the best. On the other hand, with no fat-soluble A and no fat in the diets (Groups II_3, IV_8, and VI_1), the results were slightly in favor of the 10 per cent protein diet (II_3). The non-vitamin groups (II_4, IV_6, and VI_2) show that the 10 per cent protein plane (II_4) was too low, while there was no difference between the 15 and 30 per cent diets.

To sum up, it would seem that in the main the amount of protein, whether 10, 15, or 30 per cent of lactalbumin, had comparatively little to do with the rate of development of the hind legs. There were some variations within the classes of vitamins that showed differences, but taken as a whole, one is hardly justified in placing too much emphasis upon these differences when the data for the corresponding periods are compared. This finding compares with rats where 10 per cent lactalbumin produces normal growth when properly supplemented.

If Group IV_{10}, fed the beef-oat protein diet, is compared with Group II_1, the percentage distribution is found to be in favor of IV_{10} when Period 14 is considered, but here again we are of the opinion that on the average it would be fairer to assume perhaps that there was not much difference, although when Fig. 8 is borne in mind we are almost forced to conclude that Ration IV_{10} was superior to Ration II_1. Here again it would seem that the amount of protein had little to do with the differences when we compare the data with that for Diet III_7. Furthermore, it does not seem to be a matter of vitamins or fat, for these are present in each diet. The beef or oats seemed to have carried other accessories or else the quality of protein was better adapted to the tadpole.

That lactalbumin was at least a fairly good protein for growth and development is shown from the results obtained by comparing it with a poor protein like corn gluten, which does not produce growth in rats (1, 2). Group III_5 was fed the same diet as II_1 except that corn gluten was substituted for lactalbumin. There was a distinct difference between the two in favor of the lactalbumin. When corn gluten was supplemented with an equal amount of lactalbumin protein (Ration V_1), it is seen that the

lactalbumin was able to stimulate development to some extent, but not as much as when it was used as the sole protein.

Osborne and Mendel (1) found that cystine was able to supplement a low (9 per cent) casein diet and produce the same rate of growth as a 15 per cent casein ration, without cystine. In adding cystine to a 10 per cent corn gluten diet (Group III_4), we obtained slightly better results than when no cystine was present.

From the discussion on the amount and quality of protein in the diet, it would appear that the amount of protein, from 10 to 30 per cent, had little or no influence upon the development of the tadpole, but that the quality of protein was an important factor.

(D) Influence of Kind of Carbohydrate.—In Table IV, it is possible to make a comparison of the value of dextrin and starch, Groups III_6 and II_1 respectively. It is seen that there was no difference between the two.

CONCLUSIONS.

From the data herein reported upon the size and development of the frog larvæ (*Rana pipiens*), the following tentative conclusions are made, subject to revision in the light of studies which are being started for this season, and which will be carried out with a special effort to control more carefully the matter of temperature of water and the possibilities of dominance of one individual over the other.

1. A large amount of fat in the diet was very injurious to both growth (body size) and development of hind legs. Apparently the lower the percentage of fat the better the tadpoles thrived.

2. Vitamins—the water-soluble B and fat-soluble A types—appeared to be necessary for normal growth and development. The lack of the water-soluble B type was possibly more apparent than that of the fat-soluble A. When no vitamins were present, growth and development were distinctly retarded.

3. The amount of protein incorporated seemed to play a minor part in the development of hind legs when the per cent of lactalbumin ranged from 10 to 30—whether both "vitamins," one, or none were present. On the other hand, the high protein fed tadpoles were largest (size) when both vitamins were in the diet.

4. The quality of protein was a factor which showed clearly

that it should be borne in mind. Thus, lactalbumin, and beef and oats protein when incorporated in an otherwise complete ration brought about a good rate of development, while corn gluten protein gave poor results.

5. When dextrin was substituted for starch it had no effect either on growth or development.

6. The results taken as a whole show definitely that it is possible to adjust the diet so as to alter the size of the tadpole and the rate of metamorphosis, in respect to variations in such nutrients as fat, kind of protein, and vitamins. It should be borne in mind that other factors play an important part, such as temperature and food control. Too low temperatures tend to make the tadpoles sluggish, and too high temperatures produce abnormal changes which may result in death. The tendency to consume their dead makes it difficult at times to adjust the diet absolutely.

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EXPLANATION OF PLATES.

PLATE 2.

FIG. 1. Photographic reproductions of tadpoles, showing the relative size as influenced by a high (I_1) and a low (II_1) fat diet.

FIG. 2. The diets given were both on the low fat plane. Diet II_1 contained the water-soluble B and the fat-soluble A vitamins, while Diet II_2 was lacking in the water-soluble type.

PLATE 3.

FIG. 3. Both diets contained the low percentage of fat. Diet II_1 was considered to be complete for normal growth and Diet II_3 was deficient in the fat-soluble A vitamin.

FIG. 4. Diet II_1 was compared with Diet II_4. The latter was lacking in both the water-soluble B and the fat-soluble A vitamins. These diets contained 10 per cent protein.

PLATE 4.

FIG. 5. The diets fed in this case were: complete (II_{1a}), and deficient in the vitamins (VI_2). They contained 30 per cent protein instead of 10 per cent as shown in Plate 3, Fig. 4.

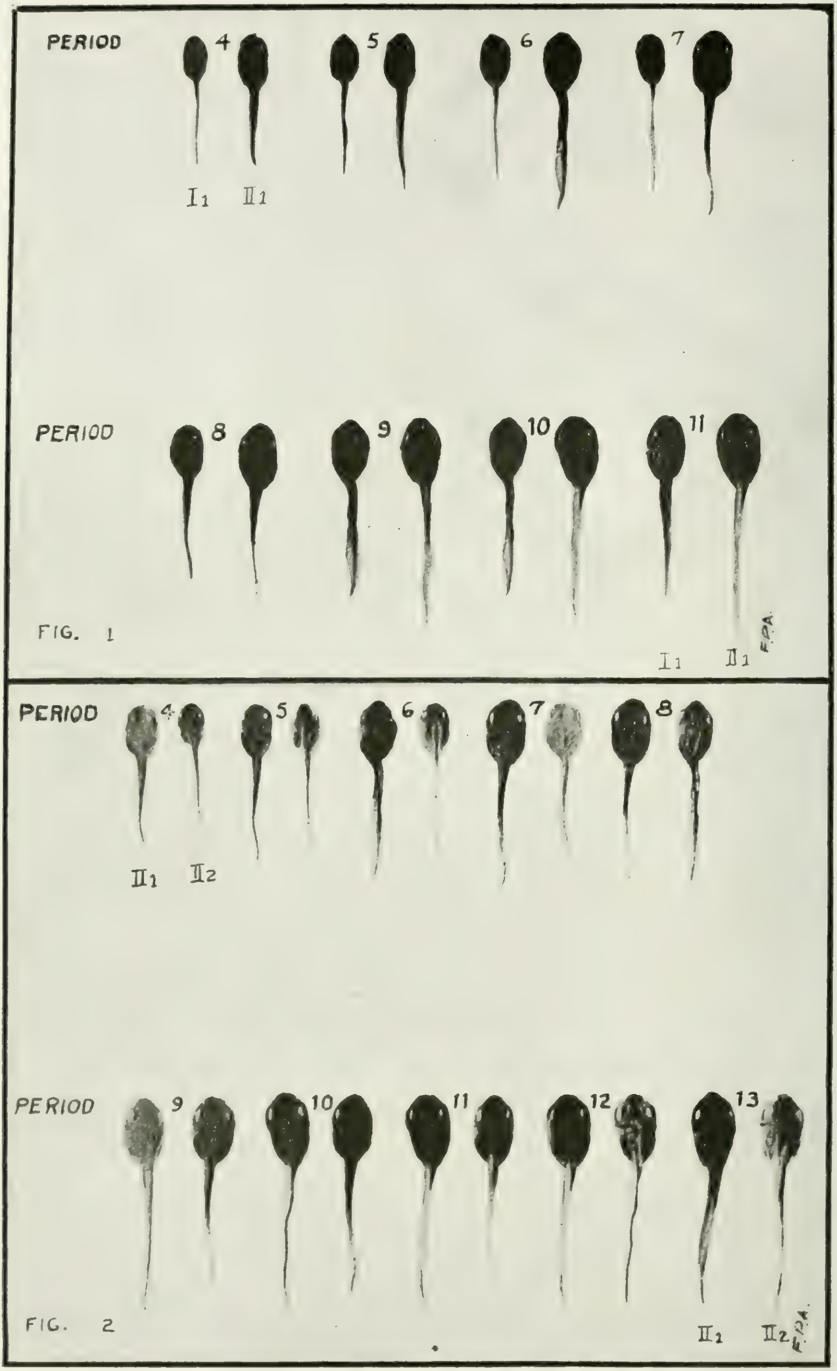
FIG. 6. Both diets were complete. Diet II_1 had 10 per cent protein and Diet II_{1a}, 30 per cent protein.

PLATE 5.

FIG. 7. The two diets differed in the kind of carbohydrate used. Diet II_1 contained starch and Diet III_6 an equivalent quantity of dextrin.

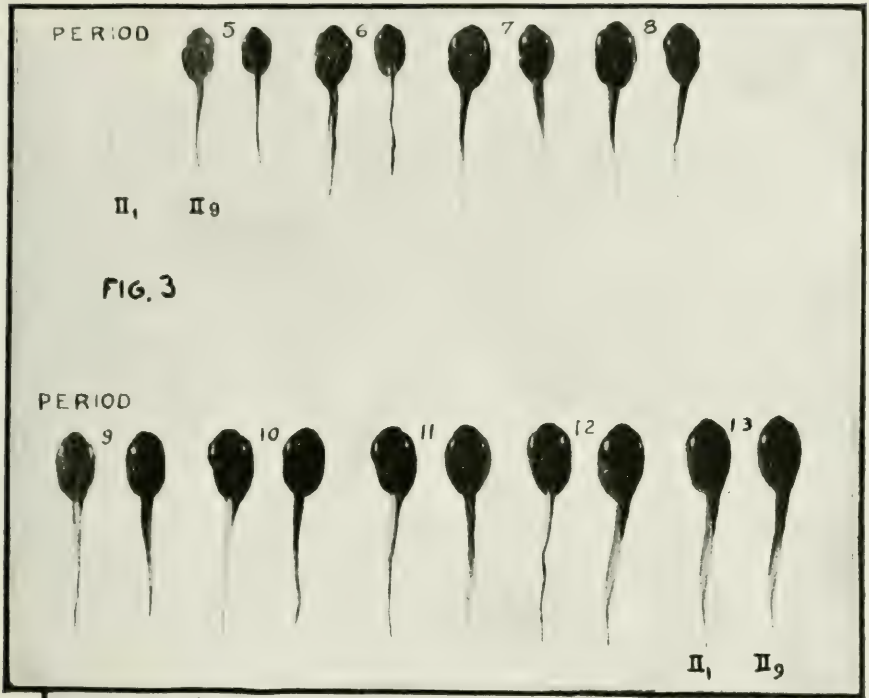
FIG. 8. Diet IV_{10} differed from Diet II_1 in having the protein derived from desiccated beef tissue and rolled oats which had been extracted with ether. The former diet contained 17.7 per cent protein and the latter 10 per cent protein. These photographs should also be compared with those in Plate 4, Fig. 6, where Diet II_{1a} contained 30 per cent protein.

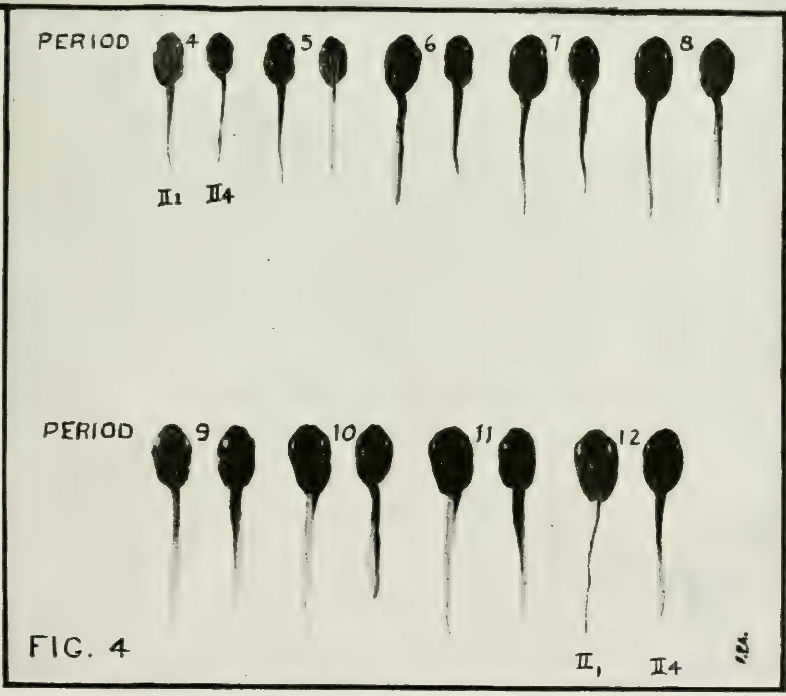
PLATE 2



(Emmett and Allen: Nutritional studies on frog larvæ.)

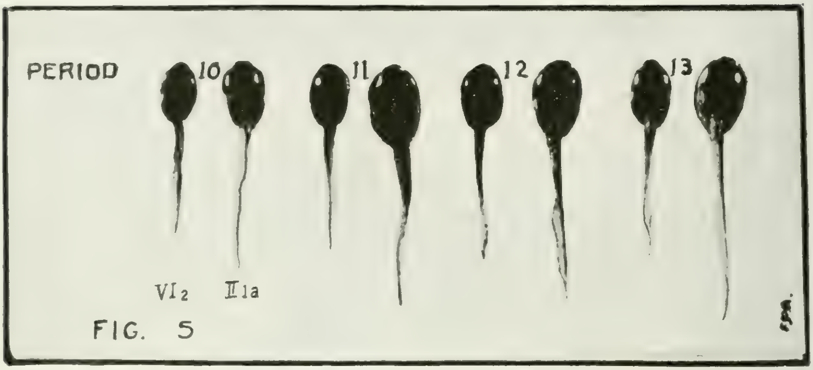
PLATE 3

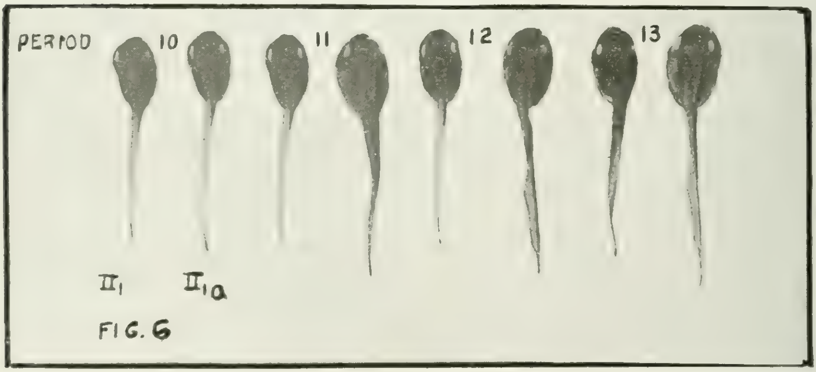




(Emmett and Allen: Nutritional studies on frog larvæ.)

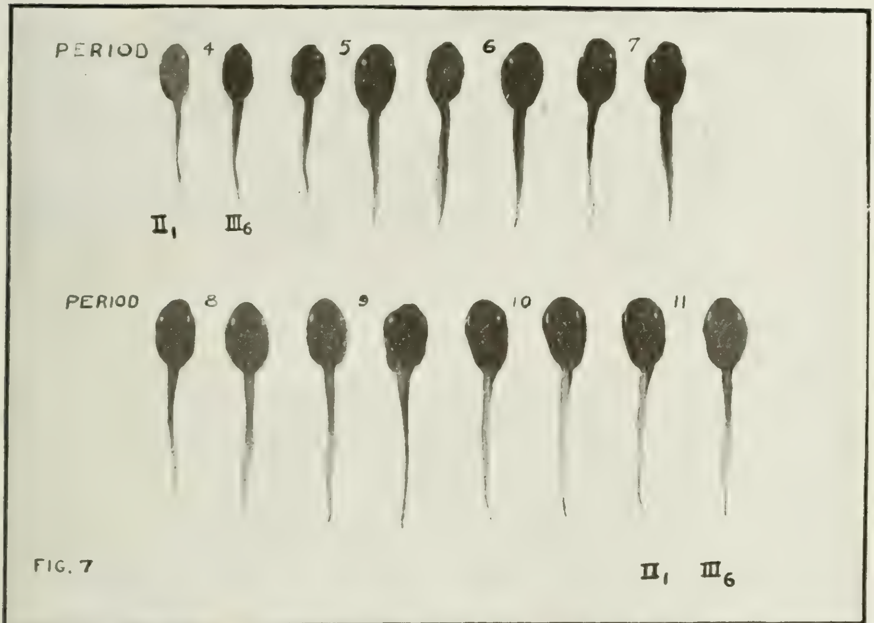
PLATE 4

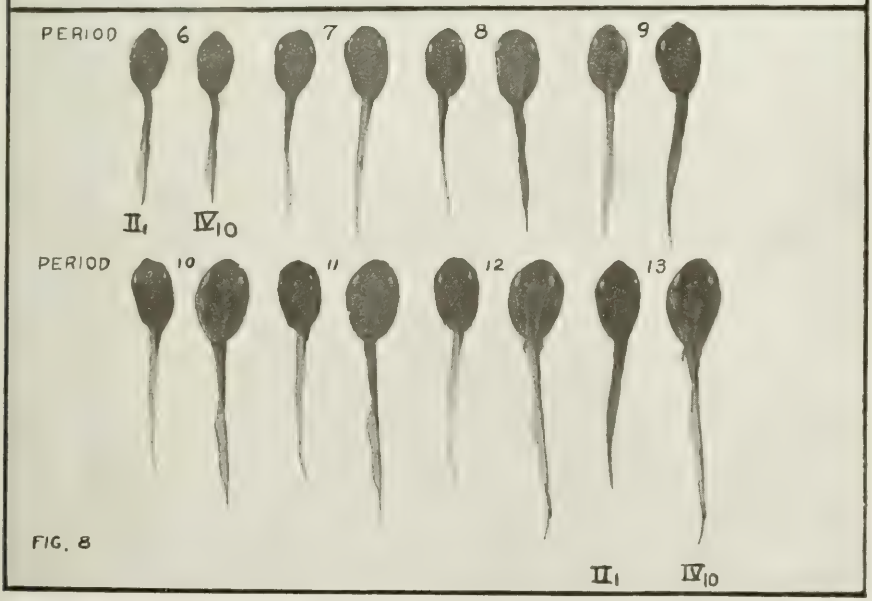




(Emmett and Allen: Nutritional studies on frog larvæ.)

PLATE 5





(Emmett and Allen: Nutritional studies on frog larvæ.)

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 212, 1919.

(Reprinted from *The Archives of Neurology and Psychiatry*, June, 1919, Vol. II, pp. 717-725.)

A CASE OF MONGOLISM IN ONE OF TWINS.*

Harold Swanberg, M.D., and H. A. Haynes, M.D.

LAPEER, MICH.

The case we wish to report is the occurrence of Mongolian imbecility in one of twins, the normal child being distinctly above the average in intelligence. Since twins are subject to identical conditions from conception to birth, and usually enjoy practically the same environment until adolescence, the rare occurrence of Mongolism in one of them permits certain inferences relative to the etiology of this form of feeblemindedness. A brief résumé of the more pertinent facts concerning Mongolian feeblemindedness is appended, together with a review of the literature of Mongolism in twins.

RESUME OF MONGOLISM.

One of the most clearly defined and best known types of feeblemindedness is that first designated as the Mongolian, Kalmuc or Tartar variety by J. D. Langdon Down[1] in 1866, so called from their facial resemblance to members of the Mongolian race. Tredgold[2] states that with the possible exception of the tongue, there are no physical signs that are pathognomonic of the condition, for all of the other anomalies present are seen in other types of feeblemindedness. However, it is the particular combination of anomalies which makes the Mongolian type so distinctive; those most important and frequently present being the small brachycephalic skull; small stature; obliquely set eyelids (hence the name Mongolian); large transversely fissured tongue;

*From the Research Department, Michigan Home and Training School (Michigan State Institution for Feebleminded), Lapeer, Mich.

[1]Down, J. L.: Clinical Lectures and Reports, London Hospital, 1866, p. 259.

[2]Tredgold, A. F.: Mental Deficiency, Ed. 2, 1914, p. 211.

short, broad hands and feet; stubby fingers and toes; peculiar shortening and incurving of little finger (Telford-Smith finger); large cleft between the great toe and the second toe; hypermobility of the joints; defective speech; dry, rough skin, especially coarse on the extremities; and poor circulation. They are happy, smiling, good-natured, amiable, active, imitative, easily managed, and nearly always imbeciles, therefore possessing a mental age which corresponds to normal children of between 3 and 7 years. Various authorities state that this group comprises from 0.9 to 10 per cent. of all feebleminded; however, the minimum figure is too low. Statistics of feebleminded institutions show that from 2 to 5 per cent. of all admissions are of the Mongolian type, and since these cases can easily be managed at home, they are probably more numerous than these figures indicate. Goddard[3] estimates there are between 200,000 and 300,000 feebleminded in the United States. If we accept these figures there are probably between 5,000 and 15,000 cases of Mongolian feeblemindedness in this country.

The etiology of at least two-thirds of the cases of feeblemindedness is hereditary, due to defective germ plasm, that is, one or both parents are feebleminded, or there is a definite history of feeblemindedness in the ancestry. Neuropathic heredity, injuries affecting the brain before or at the time of birth, traumatism to the brain in early childhood, and various pathologic conditions of the brain, as meningitis, cerebral hemorrhage, etc., during the early years of life, are the etiologic factors in the cases not covered by defective germ plasm. Mongolian feeblemindedness is an exception, however, in that the etiology is unknown. As a rule, Mongols occur in the better families, there being but the one defective, which is usually the first or last child born. The opinion of virtually all who have studied the condition is that it is due to something which interferes with prenatal development, and that the adequate cause is to be sought in the condition of the mother during pregnancy. Either uterine exhaustion, whereby the mother is not able to bring the child to full development (as seen when the child is the first born, the mother being very young, or where the child is the last born in a large family, or when the mother is nearing the menopause at the time of birth of the child) or some severe physical or mental shock to the mother which may have

[3]Goddard, H. H.: Reference Handbook of the Medical Sciences, Ed. 3, 6:384, 1916

temporarily interfered with the procreative function, seem the most tenable theories. Vital exhaustion of either or both parents is regarded by some as the etiologic factor.

LITERATURE OF MONGOLISM IN TWINS.

A careful review of the American and English and a partial review of the remaining literature pertaining to Mongolism in twins has been made. The following cases have been reported:

Hjorth[4] reports the occurrence of Mongolian feeblemindedness in twins, each presenting the specific characteristics. The father was a day laborer and was 41 years of age at the birth of the children, the mother 42. These children were the eighth and ninth in a family of ten. This is the only case reported that we have been able to find of the occurrence of definite Mongolism in both twins.

McKee,[5] in reporting fourteen cases of Mongolism of which one was in twins, states only that "one baby was the smaller of twins." Nothing is mentioned concerning the other child, but it is evident that it was not a Mongol.

Shuttleworth[6] states, "Among my illustrations I have the pleasure of showing you two remarkably fine photographs of twin children (boy and girl) from Melbourne, one (boy) normal, the other (girl) a Mongolian imbecile, for which I am indebted to Dr. A. Jeffreys Wood of that city. . . . The case of twin pregnancy with offspring of different sex, the boy normal, the girl a Mongolian imbecile, is unique in my experience."

AUTHORS' CASE.

Family History.—The parents of our patient are both collegiate graduates and highly respectable people. The father, now 50 years of age, married at 26, but on account of very poor health at that time, had to give up a confining position to get out of doors. His condition was diagnosed as pleurisy, tubercular origin suspected, and a grave prognosis given. He took up farming in order to build up his health and continued at this for many years. At present he enjoys fair health and his wife describes him as being "tall, slim, very nervous and lacking vitality." The mother, at present 53 years of age, has always enjoyed good health. She married at 29. As a result of this marriage there have been five children (two being twins) and two miscarriages. The oldest child, a girl of 23 years, is in fair health; the next, a girl of 21, is in excellent health. Both girls are brilliant students in one of

[4] Hjorth, Bodil: Nyt Tidsskrift for Abnormvaesenet, No. 9, 1906.

[5] McKee, J. H.: J. Psycho-Asthenics 13:43, 1908-1909.

[6] Shuttleworth, G. E.: Brit. M. J. 2:661 (Sept. 11), 1909.

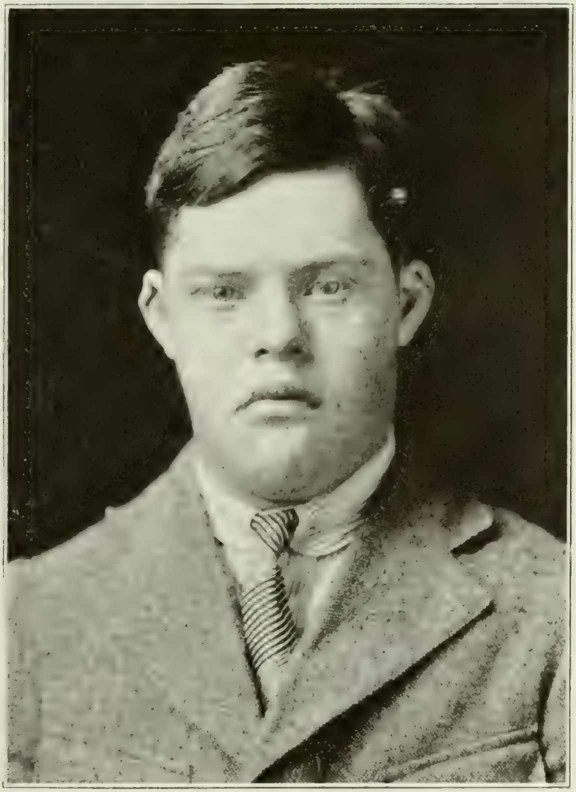
our large universities. The next child is a boy of 20, who has tuberculosis. At present he is farming as his father did when similarly ill. The next pregnancy resulted in a miscarriage. Following this the twins were born, now 17 years of age, one being normal, the other a Mongolian imbecile. Another miscarriage followed the birth of the twins.

We have been fortunate in securing an excellent genealogy of both the paternal and maternal sides of this family. In nearly 200 known blood relatives on both sides, representing five generations for the paternal and four for the maternal side, no cases of feeblemindedness have been established, except the one under consideration. There is one case of insanity—a religious mania—which affected a sister of our child's grandmother on the maternal side. There is one case of alcoholism which occurred in the maternal grandfather. The only tuberculosis is on the paternal side. Our patient's father in all probability had tuberculosis, the brother has it, an uncle recovered from it and the great-grandmother died of it. The paternal grandfather and grandmother died at about 80 from senility. The maternal grandfather, who was alcoholic, died suddenly at 45 from "heart trouble," and the grandmother at 78 from "anemia." Two other instances of twinning are seen in the family, both on the maternal side. An uncle of our case was the father of twin boys who were born prematurely, living but a few hours. The great grandfather had brothers who were twins, and lived to old age. The brothers and sisters of our patient have had excellent school records and the majority of the relatives are above the average in intelligence, two uncles and one aunt being college professors.

Mongolian Twin.—History.—The patient was a boy, aged 17, whose father was 33 and mother 36 at the time of birth of twins who were the last born of a family of five. Mother had no unusual events during the pregnancy except that she frequently fainted. During previous pregnancies she was also subject to syncope, but with the twin gestation it was much more frequent. During the latter part of the pregnancy she suffered from severe pains in the upper part of the abdomen, presumably due to pressure from the fetal parts. The father was in fair health at the time of conception. The labor was normal, no instruments used, and the mother did not suffer more at this time than during her previous deliveries. The normal girl was the first born, weighing 5¾ pounds. She was a "blue baby" according to the mother, the "heart not closing" until the following day. The Mongolian boy was born a half hour after his normal sister. He appeared very pale, and weighed 6½ pounds. Even at birth he had the Mongolian facies, and the fingers were short and chubby in contrast to the long and slender ones of his twin sister. The integument remained soft and did not assume its normal firmness until 3 years of age. He was nursed by the mother and was a very good baby who seldom cried. Teething began at about 8 or 9 months, and was very irregular. He did not commence to walk until 3 years old and did not talk until 6. He never had any bad habits, and at present is quiet, good-natured and easily managed. At 2 years of age he had scarlet fever with otitis media as a complication, which left a chronic discharging ear; at 3, measles and pertussis; and later adenoids, which were removed. There is no history of convulsions. His schooling has consisted of two years at kindergarten, and three years of special class work for backward children in the public schools. From his 5th to 17th year he was on thyroid treatment most of the time. The parents believe it has helped him and state his "tongue becomes thicker" and voice more imperfect when he is not taking the drug.

Physical Examination.—His height is 5 feet, and his weight 122 pounds. Musculature and nutrition are good. The head is somewhat small, but of normal shape, the greatest antero-posterior diameter being 6¾ inches, transverse diameter 5¼ inches, and circumference 20½ inches. The fontanelles

are closed, the sutures are not palpable, the hair of the head is very abundant, coarse, straight and of red color. The face is freckled, normal symmetry, oval shape and covered with fine hair. The nose is short with nostrils tending to look forward. The upper part of the lobule of each ear is somewhat deformed, but hearing is good. The eyes are slightly obliquely placed, the pupils are equal in size and react normally. The lids appear normal, no nystagmus or squint, and vision is about 20/40 for each eye. The teeth are in fairly good condition, gums normal, palatal arch high and narrow, tonsils small, no adenoids, and lips normal. The tongue is very large, and transversely fissured, papillæ enlarged. The mouth is usually partially opened and



Authors' case. Mongolian imbecile, aged 17.

the speech imperfect. There are no lymph glands palpable in the neck, the thyroid is not enlarged although the neck is large, the circumference being 14¾ inches. The respirations are regular, abdominal type and 20 in frequency. The lungs and heart are normal. The abdomen is protuberant with tendency to an umbilical hernia, and there is a lordosis of the lumbar spine. The genitalia are normal, with a normal amount of pubic hair which has a semi-feminine distribution. The hands and feet are of clumsy appearance. The fingers are short and stubby, but the thumbs and little fingers are not

short, nor are the latter abnormally curved as often described as typical of Mongols. There is a large cleft between the great toe and its adjacent fellow. There is a great laxity of all the ligaments resulting in hyperextensibility of the joints. The skin is normal except that covering the hands, which is thick and rough (not due to manual work). There are no varicose veins. The knee jerk and cremasteric reflexes are normal. The grip is good, gait normal, no paralyses or tics, and coördination normal. The pulse is 74 and ocular pressure only lowers it to 69. The systolic blood pressure in the sitting posture is 135, diastolic 80. The temperature is normal and the hemoglobin, by the Tallqvist test, 80 per cent. A complete urinary analysis was not made, but the specimen was clear, straw color, characteristic odor, specific gravity 1.023, and no albumin present. The Wassermann reaction of the blood serum was negative.

Normal twin sister of Mongolian imbecile.

Psychologic Examination.—(The psychologic examination was made by Miss Z. P. Buck, A.M., resident psychologist, Michigan Home and Training School.) The patient could not correctly repeat a sentence containing twelve syllables, failed to distinguish orange from yellow, did not know which was his left ear, could not describe pictures, could not repeat five digits, or repeat three digits backward, could not count backward from twenty to one, could not give similarities between two simple things, could not define objects in terms superior to use, did not know day of week, month or year, could not discriminate between various weights, could not make simple change such as 4 from 10 or 12 from 15 cents, could not use three words in a sentence or give three words that would rhyme with given simple words, and his

vocabulary was extremely poor. A more detailed psychologic examination was made and the following is the psychologist's report:

"By the Terman Test our case has a mental age of 6 years and 6 months, which gives him a retardation of 9 years and 6 months. The Seguin Board also gives him a mental age of between 6 and 7 years (Doll's Standardization).

"Throughout all tests the patient shows an ability to discriminate better than that of the average child of his mental age (Seguin, Card Dealing and Healey Picture Puzzles). His fatigue curve is irregular but remarkably well sustained, as the curves show. It will be seen that even at the end of thirty minutes he is still making a higher record than during the first minute. The attention is, in general, poor. He is easily distracted and there is a limited amount of fluctuation. The powers of memory, apprehension, and apperception are also poor. In reasoning, with the exception of the comprehension question designed for a child of 8 years, and the most elementary addition problems, there is a complete failure. The will power in general is very good but will doubtless be used without reason. He responds immediately to praise and works with determination and thought such as he is capable of. The patient shows the usual Mongolian power of imitation. There is also a very evident personal pride. He carries himself erect and with chin up, frequently expands his chest to its greatest capacity and looks about for praise. He shows his muscles and readily undertakes anything that will increase his physical strength. Asked what he was doing in the cottage he straightened up, felt his muscle, and replied, 'growing'."

To summarize: The patient shows (1) good discrimination; (2) splendid endurance; (3) from fair to poor attention; (4) poor apprehension; (5) poor apperception; (6) poor memory; (7) very poor reasoning; and (8) a strong but irresponsible will power.

Normal Twin.—The twin of the Mongol is a normal, healthy girl. She has always enjoyed good health and in no way resembles her defective brother. She is 5 feet 5 inches tall and weighs 110 pounds. Her hands and feet are long and slender, her hair is fine and tongue normal. Both physically and mentally she has progressed as any normal child. She started in her school work at 7½ years of age. The first and second grades were completed in one year, as were also the sixth and seventh grades. She was graduated from high school at the age of 17. Her school record has been excellent and if we were to judge her mental ability by this, she could easily be placed above the average in intelligence. In her four years at high school, she never received less than 90 in any subject, and was always among the leaders of her class; her grand average for the four years being 93.4 per cent. Her best subject was mathematics, in which her average was 95.5 per cent for the four years. This average included all the courses given in that subject.

COMMENT.

Many cases of twin births in the feebleminded have been reported. In most instances both children have been feebleminded, but in some cases one twin has been feebleminded while the other enjoyed a normal mentality. Goddard[7] reports four such cases. This latter phenomenon can be easily understood if we accept Goddard's[8] explanation. He states that normal-mindedness is, or

[7]Goddard, H. H.: Feeble-Mindedness: Its Causes and Consequences. Macmillan Co., New York, 1914, p. 525.

[8]Goddard, H. H.: Ibid., p. 548.

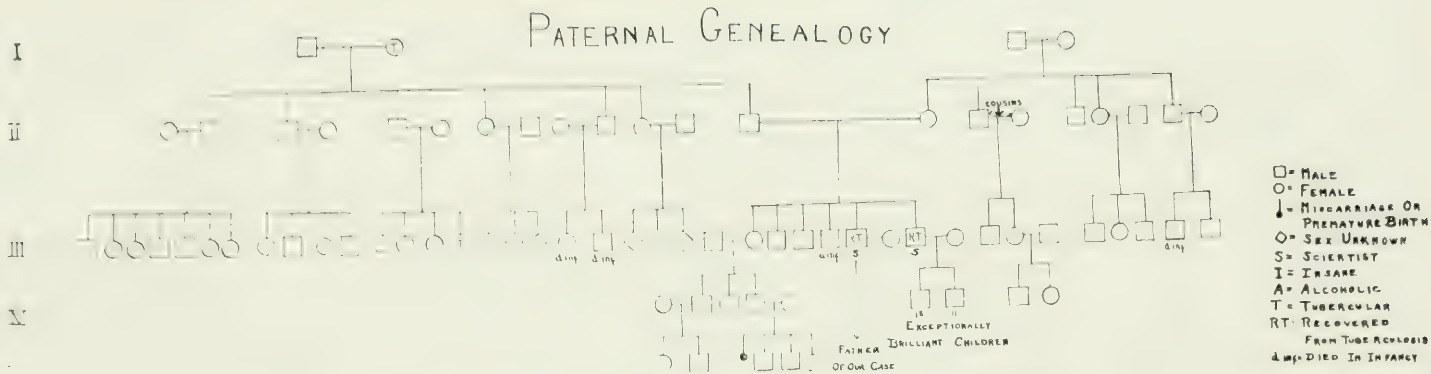


Chart 1.—Genealogy of paternal side of authors' case of Mongolian imbecility.

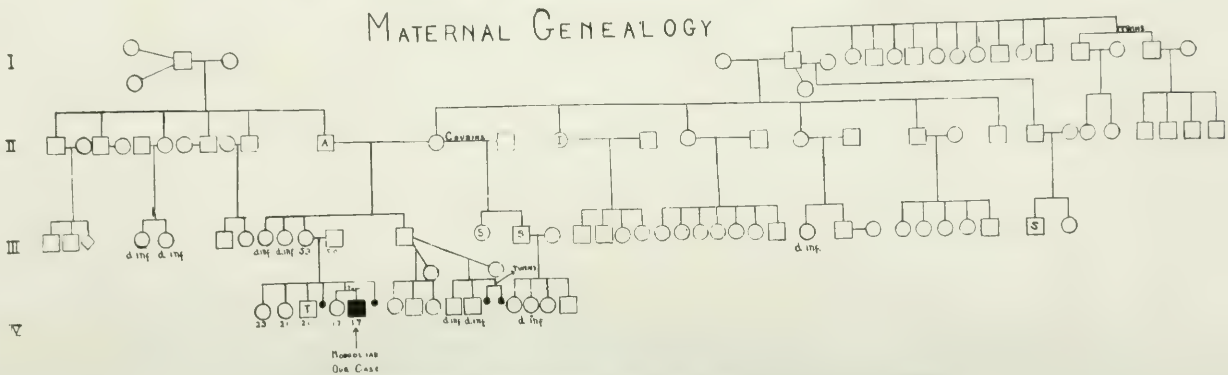


Chart 2.—Genealogy of maternal side of authors' case of Mongolian imbecility.

at least acts like, a unit character; is dominant and is transmitted in accordance with the Mendelian law of inheritance; feeble-mindedness also behaves like a unit character, is recessive, and is, like normal-mindedness, transmitted in Mendelian proportions. If feeblemindedness is transmitted according to Mendel's law, as there is very good reason to believe, it is easy to explain the difference in the mentality of the twins. Goddard[9] states: "We have two ova, fertilized by different spermatozoa, each of them subject to whatever possibilities the conditions of the chromosomes warrant. In the one case a 'defective' spermatozoan has fertilized a 'defective' ovum with the resulting defective offspring. In the other case a normal spermatozoan has fertilized a normal ovum, or else one of the germ cells has been normal and the other 'defective,' in either case resulting in a normal offspring."

However, the above discussion does not apply to Mongolian feeblemindedness, for it is an exception, in that it is not hereditary and does not, therefore, follow Mendel's laws. In this respect it is similar to those forms of feeblemindedness that are due to accidental causes acting before, at, or shortly after birth.

To our knowledge no Mongol has ever become a parent. The genital organs of Mongols appear to develop normally, but the sex sense seems to be in abeyance, and some hold they are sterile. Their degree of mental deficiency is so pronounced that marriage is unthought of. This eliminates entirely the possibility of any light being thrown on the etiology by study of the offspring.

There are no grounds for explaining the condition on the basis of birth traumatism, for the physical characteristics of Mongols are already formed before birth. Moreover, in our case the birth was not only normal in every way, no instruments being used, but the normal twin was the first born and would, therefore, be the most likely to suffer from birth traumatism due to the crowded pelvis.

The mother suffered more during this pregnancy than any other, but this is to be expected in twin gestation. In twin pregnancies the mother is more subject to toxic conditions, etc., than when but one fetus is present.

As to the father's health at the time of conception little can be said. He was not robust, but was in much better physical con-

[9] Goddard, H. H.: Ibid., p. 526.

dition than at the time of conception of his first child, when he was in all probability tubercular. As both parents are total abstainers, any thought of alcoholism can be eliminated.

Recently there has been an endeavor to explain the etiology of Mongolism on the grounds of congenital syphilis. As a result of serologic tests in thirty-eight Mongols, Stevens[10, 11] reports a positive Wassermann of the blood serum in 21 per cent., positive Wassermann of the spinal fluid in 18.4 per cent., and in 94.7 per cent. a positive reaction to Lange's gold chlorid test. He states that, "the serologic tests seem to demonstrate beyond question the condition is a result of syphilitic infection." McClelland and Ruh[12] answer Stevens' contentions by criticizing his method of interpreting the tests under discussion. In serologic tests of thirteen Mongols they found negative Wassermann reactions in the blood and spinal fluid and negative Lange's gold chlorid tests in every instance. They state, "from careful anamneses, physical examinations and the laboratory tests now available, it cannot be stated at the present time that Mongolism is due to congenital syphilis." Goddard[13] states that of twenty-eight Mongolians examined at the Columbus Institute, Ohio, 17.8 per cent. gave a positive Wassermann reaction (presumably of the blood serum); and at the Keller Institution in Denmark, no Mongolians gave a positive Wassermann reaction. Of his own cases at Vineland, where two series of examinations were made, and both blood and spinal fluid tested, he states, "in neither of these was the percentage of positives noticeably high." Quoting further from Goddard:

> "I have not been able to find any conclusive proof that syphilis in the parents causes feeblemindedness of any type, to say nothing of Mongolism. . . . To these difficulties in the way of regarding syphilis as the cause of Mongolian imbecility, I think may be properly added the further one that there is no correlation between the instance of Mongolism and syphilis. Mongolian imbecility is, relatively speaking, very rare; syphilis is far from rare. If syphilis is the cause of Mongolian imbecility, are we not compelled to conclude that the latter would be vastly more prevalent than it is?"

[10]Stevens, H. C.: Mongolian Idiocy and Syphilis, J. A. M. A. 64:1636 (May 15), 1915.

[11]Stevens, H. C.: The Spinal Fluid in Mongolian Idiocy, Ibid. 66:1373 (April 29), 1916.

[12]McClelland, J. E., and Ruh, H. O.: Syphilis as a Factor in Mongolian Idiocy, J. A. M. A. 68:777 (March 10), 1917.

[13]Goddard, H. H.: Syphilis as an Etiologic Factor in Mongolian Idiocy, Ibid. 68: 1057 (April 7), 1917.

The only suggestion of syphilis in our case is the history of miscarriage before and after the birth of the twins. An examination of the blood serum of the defective twin gives a negative Wassermann. While it is true syphilis would explain why one child was affected and not the other, the possibility of syphilis explaining all cases of Mongolism is very remote, as the work of McClelland, Ruh and Goddard, quoted above, would indicate.

In conclusion, we may state that there is apparently nothing in the history of the mother, father or family to account for the occurrence of Mongolian imbecility in this twin. The defective child had the stigmata of the condition at birth; hence it is, no doubt, due to some congenital factor. Mongolism occurring in one of twins only further convinces us of the obscure etiology of this affliction. Granted that it is due to an unknown congenital cause, we are at a loss to explain why one child is so affected and the other escapes entirely.

**Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 213, 1919.**

(Reprinted from *Rhodora, Journal of the New England Botanical Club*, Vol. XXI, 1919.)

TSUGA AMERICANA (MILL.) FARWELL: A FINAL WORD.

Oliver A. Farwell.

(Department of Botany, Parke, Davis & Co., Detroit, Michigan.)

In *Rhodora* for October, 1918, pages 185-8, Mr. Tidestrom argues for the retention of the name *Tsuga Canadensis* (L.) Carr. for the Hemlock Spruce on the grounds that Linnaeus, in 1739, assisted in writing up the description of the plant for Gronovius's *Flora Virginica*, 1743, and hence was familiar with the species and therefore the element Linnaeus knew should be considered as the type; also that the word *submembranaceis* of the description excludes the White Spruce from consideration. He fails to prove, however, that the White Spruce was not equally known to Linnaeus; he only supposes that it was not. He says: "That Linnaeus meant that his *P. Canadensis* should stand for a Spruce as we understand this genus is out of the question." Since Linnaeus included the "Spruce" as an element of his *Pinus Canadensis* it is rather astonishing, to say the least, to learn, "That Linnaeus meant it, is out of the question." Philip Miller, a contemporary of Linnaeus and a botanist of no mean ability, ranking perhaps in his day as second only to the distinguished Swede, and one who probably knew as much as any about the then current concept of species, certainly understood *Pinus Canadensis* Linn. to be the White Spruce. Note the description of each:

Pinus Canadensis, Linn.	Abies Canadensis, Miller.
Pinus foliis solitariis linearibus obtusiusculis submembranaceis.	Abies (Canadensis) foliis linearibus obtusiusculis submembranaceis.

With the exception of the word *solitariis* these descriptions are identical and it is *self-evident* that Miller *adopted the specific name and technical description from Linnaeus;* also that if the word

submembranaceis excludes the White Spruce in one instance it must in the other also. *Abies Canadensis* Miller as to name and technical description, but not as to plant, is a *pure synonym of Pinus Canadensis* Linn., *thus leaving Miller's plant nameless;* yet Mr. Tidestrom accepts *Picea Canadensis* (Mill.) Britt. If *Pinus Canadensis* Linn. (*Abies Canadensis* Mill. as to name bringing synonym) is legitimately construed as the Hemlock Spruce, then the *nameless plant* of Miller, the White Spruce, must be given a different appellation than the one by which it is now known, since two species can not be given the same specific name when based upon the same earlier binomial. One or the other must drop the specific name *Canadensis:* if it is to be the Hemlock, then its name should be the one heading this article; if the White Spruce, the name for it should be PICEA GLAUCA (Moench.) Beissn. (*Pinus glauca* Moench., Verz. 73, 1785.)

For my part and with all due respect to Mr. Tidestrom, I fail to see that he has thrown any new light upon the subject; he has not shown the *determining incident occurring after 1753* that induced Linnaeus to create a new binomial or species, *if it were not,* as previously maintained by me, the *publication of Miller's plate and description.* If Linnaeus did not know the White Spruce, the plate of Miller illustrating it was second only to an actual specimen in hand, and therefore he became through studying the figures as familiar with the Spruce as he could have been with the Hemlock from an examination of the Clayton fragmentary twig, some twenty odd years previously; he was at the time (when Miller's *figures* were brought to his attention) probably engrossed with the production of the 2nd Ed. of the Species Plantarum; his study of the Hemlock was brought to mind; he saw a greater resemblance in it to the Spruce than to the Balsam Fir; it was, therefore, taken out of *Pinus Balsamea* and placed under his new species, *P. Canadensis,* where it "should not be considered as necessarily belonging to the species, but that it was possible that such was the case;" having brought these two species together, that he used the old description of his own rather than that of a rival author was perfectly natural, and quite understandable. Since, however, the plate of Miller is the determining factor in the creation of *Pinus Canadensis,* it should be considered as the type.

**Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 214, 1919**

(Reprinted from *The Veterinary Alumni Quarterly*, Vol. VII, No. 1, June, 1919.)

ANAPHYLAXIS IN VETERINARY PRACTICE.*

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Anaphylaxis is a term coined by a French investigator, Charles Richet, whose investigations as far back as 1902 really laid the foundation for our present knowledge of the subject. The discoveries of Arthus and the observations of Von Pirquet and Schick were among the contributions to our earlier knowledge of anaphylaxis. Etymologically the word anaphylaxis is built up from two Greek words, *ανα* (against) and *φύλαξ* (a guard). To-day the word really has a double meaning, (a) the phenomena or symptoms of intoxication exhibited by a sensitized animal following the second injection of a foreign protein after an appropriate interval, or (b) the state of being sensitized to a foreign protein.

In bringing about an anaphylactic state, there are three distinct steps: (1) sensitization; (2) incubation; and (3) intoxication. Proteins, of which there is an endless variety, seem to be the only substances that are capable of bringing about the anaphylactic state, and these proteins must be foreign to the animal receiving them, or anaphylaxis will not result. Further, the protein substance must be unaltered, in a soluble state, and must reach the blood or lymph of the animal, for the latter to become sensitized. Blood serum and hemoglobin, erythrocytes, leucocytes, milk, egg albumen, organ extracts, spermatozoa, pollen granules, etc., are all substances that may be employed to demonstrate anaphylaxis, and at times only surprisingly small amounts of these substances are necessary to demonstrate the phenomena of the reaction.

All species of animals appear to be capable of sensitization. Most observations have been made on the guinea-pig, rabbit and dog, although the literature on the subject contains references to

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*Read before Northwestern Ohio Veterinary Medical Association, Toledo, Ohio, February 27, 1919.

other animals, including the horse, cow, goat, sheep, pig, rat, white mouse, frog, and various birds. Of the laboratory animals the guinea-pig is probably the best for demonstration purposes, and the injections of the protein should be made subcutaneously, intravenously, or intraperitoneally.

The incubation period in anaphylaxis is quite similar to the incubation period of an infectious disease. The period varies in different species, the method or site of injections, and the quantities of the substance used. In anaphylaxis the period of incubation may be said to be the interval between the first injection of the protein material and the earliest appearance of anaphylactic shock following the second injection of the same protein material. The period varies from one week to four.

Intoxication is usually manifest by functional disturbances of various organs, varying considerably with different species. For instance, in the anaphylactic guinea-pig the lungs seem to be the center of the disturbance. In the rabbit there are profound changes in the cardiovascular apparatus. In the dog we have both circulatory and gastro-intestinal symptoms, while man frequently shows marked cutaneous eruptions. Animals and man may continue to be sensitive for a number of years following the initial injection of the foreign protein. The character of the anaphylactic reaction is fairly constant for a given species, irrespective of the protein used—that is, different proteins invariably produce the same symptoms in the same species.

That form of anaphylaxis known as "serum disease" is a phenomenon that has undoubtedly been of more concern to the practitioner of human medicine than to the veterinarian. However, in view of the fact that the underlying principles of the phenomenon are identically the same in both man and lower animals, a brief review of the subject might be apropos at this time. The term "serum disease" has been given to anaphylaxis, because the first experiences with anaphylactic shock were had in connection with the administration of antidiphtheric serum, but before the exact nature of the trouble or the conditions surrounding it were known. As the veterinarian's experiences with anaphylaxis are quite likely to be in connection with the administration of serum, we will consider the phenomenon from this standpoint.

Blood serum is rich in protein substances. When proteins are

ingested, in the form of food, the digestive juices and their enzymes break up these complex protein substances into much simpler bodies before their absorption into the blood, and no untoward reaction of any kind is the general rule. There are exceptions, however, in the cases of certain individuals, who invariably exhibit a chain of symptoms following the ingestion of a certain food, one of the most common being the skin eruption, in the form of hives, exhibited by persons a short time after eating the food to which they are peculiarly susceptible.

Anaphylactic reactions are most typical when they follow the parenteral introduction of protein substances. By parenteral we mean the introduction by some route other than the digestive tract, for instance, subcutaneously, intravenously, or intramuscularly. That the substances which will produce anaphylaxis, under certain conditions, are in themselves harmless, has been proven many times. On the other hand, it is the condition of the animal or the person, with reference to the protein, which results in anaphylactic shock when the protein is injected.

For instance, we may take a healthy, normal dog and inject subcutaneously 5 mils of normal horse serum. No untoward symptoms follow, nor is any reaction manifest, locally or otherwise. We keep the dog under observation for some time, and again inject 5 mils of normal horse serum. Almost immediately the dog begins to show signs of distress, and we have a typical anaphylactic reaction. If we take another dog, and repeat the above experiment, but substitute normal dog serum for the normal horse serum, no anaphylactic reaction will follow. This is because a foreign protein is required to bring about the anaphylactic condition.

By way of explanation it may be said that the first dog was sensitized by the first injection of the foreign horse serum. The second dog was not thus sensitized, because the serum was homologous (from the same species). That an incubation period is required for the complete sensitization of the animal can be demonstrated very easily, by giving two injections, closely following one another, in which case no reaction follows. However, if we allow an interval of time to elapse, after the second injection, before giving a third, we will find our animal sensitized and capable of reacting anaphylactically when the third injection is given.

The accepted explanation of this phenomenon assumes that following the initial introduction of a foreign protein, the body reacts and specific antibodies are formed. In this case these antibodies exist in the form of ferments, which have the property of splitting up the protein molecules into a number of parts. Some of these are poisonous and the symptoms which characterize anaphylactic shock are attributed to the tonic actions of the split-products of the protein molecule.

Now, how does anaphylaxis concern the veterinarian? The multiplication of serums for prophylactic and curative use, in the control and treatment of the various infectious diseases of animals, brings the subject very close to home. At the present time we have the following serums being used more or less extensively by veterinarians:

Antitetanic serum (tetanus antitoxin)
Anti-influenza serum
Antistreptococcic serum
Anti-white scours serum
Anti-distemper serum (canine)
Anti-hemorrhagic septicemia serum
Anti-blackleg serum
Anti-hog cholera serum
Anti-anthrax serum
Anti-rinderpest serum
Anti-abortion serum

With one exception, in the case of anti-hog cholera serum, either horses or bovines are used in the production of these serums. Anti-hog cholera serum is produced exclusively from hogs, and is used exclusively on hogs, and therefore may be dismissed from this discussion, in so far as any dangers in connection with its use are concerned. Anti-rinderpest serum may also be dismissed, as it is produced from and used exclusively for the treatment of bovines.

Antitetanic and anti-influenza serum should present no difficulties, in view of the fact that these serums are produced exclusively from horses, and their use is practically confined to the same species. Antidistemper serum (canine) should present no difficulties, but for a different reason. The serum is produced

from horses, but used on dogs, therefore a foreign serum. However, it is practically the only serum that is used to any extent on dogs, and when it is used should cause no trouble, owing to the fact that when more than one dose is necessary, they are given close enough together to practically eliminate any danger of anaphylaxis.

Antistreptococcic serum is produced from horses and largely used in the treatment of diseases of this species. The antigen used in its preparation consists of various strains of streptococci isolated from lesions in horses, believed to be due primarily to streptococcic invasion. We have conditions in other species that are probably due to streptococci, certain forms of mastitis, for instance, and for this reason there would be a good argument for using antistreptococcic serum in this condition, especially if it were polyvalent and in its preparation strains from bovine sources had been used.

For these reasons, therefore, antistreptococcic serum would fall into the same class with anti-white scours serum, anti-anthrax serum, anti-blackleg serum, anti-abortion serum, and anti-hemorrhagic septicemia serum, in view of the fact that these serums are produced from horses and largely used in the treatment of cattle. The danger lies, not in the case of any one serum, but in the fact that all are immune horse serums that may be used at different times in treating an animal of the bovine species.

It is not my intention to imagine a condition which does not exist, nor to magnify or exaggerate a danger which, at the worst, is small. On the other hand I will be content to state the facts in the case and cite a few instances, or combinations of circumstances, which might lead to embarrassment.

Suppose, in a certain herd, it is customary to give each calf, at birth, a prophylactic dose of anti-white scours serum. Some months later anthrax makes its appearance in the herd, and it is decided to give each animal in the herd a dose of anti-anthrax serum. There is some danger of inviting anaphylactic reactions, unless the veterinarian takes precautions to prevent this very thing from happening. This can be done very easily by administering what is called a desensitizing dose of serum. This is a small dose, from 0.5 to 1.0 mil, administered as a sort of "feeler." If

the animal is sensitized, the reaction following will be very mild, and in a short time the desired larger dose may be administered without danger. This procedure is very frequently followed by the practitioner of human medicine, who really faces greater difficulties in this respect than the veterinarian, because the serum is almost invariably foreign to the patient, and the latter may have been sensitized by the previous use of a certain serum administered at a prior illness. Various methods have been advocated for eliminating the dangers of anaphylactic shock, following the injection of serum. One of these that is undoubtedly important is to inject the serum very slowly when giving it intravenously.

In anthrax-infected districts it is quite often customary to vaccinate the animals once a year, or oftener in case of a bad outbreak. If the serum-simultaneous method is used, there is some danger of inviting anaphylactic reactions in the cattle and sheep. Several years ago Alexandresca and Ciucia, working in Roumania, absolutely prevented anaphylactic reactions in cattle by administering a desensitizing dose of serum, while in the cattle that were not desensitized more than 10% exhibited anaphylactic reactions. Other methods that have been tried in the attempt to prevent these reactions are: heating the serum, the addition of hydrochloric acid to the serum, rectal injections of the serum, and various methods of combining the serum to be used with other sera.

There are several animal diseases, the etiolgy of which has not been satisfactorily determined, which various authorities have at times believed to be of an anaphylactic nature. Parturient paresis (milk fever) is one such disease, but to accept it as a form of anaphylaxis we must modify our ideas of the condition somewhat. It is an established fact that in the same species the symptoms are always about the same, irrespective of the substance or material bringing about the anaphylactic reaction. The tuberculin and mallein reactions are anaphylactic in nature. One of the most common conditions in human beings believed to be of an anaphylactic nature is hay fever.

Hadwen and Bruce[1] have described cases of anaphylaxis occurring naturally, suspected of having been caused by warbles. These having been crushed subcutaneously, through an injury, the material liberated was sufficient in quantity to produce shock. Their experiments with injections of aqueous extracts of Oestrus ovis,

Hypoderma bovis and Gastrophilus equi larvæ indicated that the hosts of these parasites were sensitized, and would react when intravenous injections were made, using aqueous extracts of the animals' own larvæ. Their interest in the subject centered around the etiology of infectious anemia of horses. The Seyderhelms[2] and Ries[3] believed that this disease was caused by toxic substances elaborated and given off by Gastrophilus larvæ. Van Es and Schalk[4] showed very conclusively that such was not the case, but that the results obtained by the European investigators could be attributed to anaphylaxis. Williams and Hagan[5] have given a good description of the anaphylactic symptoms shown in young heifers, following the fourth injection of serum, at intervals of 17, 15 and 12 days, no reaction having been noted at the second and third injections.

Anaphylactic symptoms.

BOVINES:

Uneasiness or excitement, followed by depression, collapse, prostration and opisthotonos; trembling, most marked over the hind quarters and shoulders; profuse lacrimation with swelling (edema) of the eyelids; mouth open and tongue pendulous; swelling of the anus and vulva; cyanosis; extension of the legs, which are rigid but flexible; swelling of the udder; respiration increased, after initial, temporary decrease in rate; stertorous breathing; sweating; increased peristalsis, with passage of mucus, feces and flatus; micturition.

HORSES:

Uneasiness and trembling of certain groups of muscles; sweating; symptoms of colicky pains; tenesmus; defecation at frequent intervals; nasal discharge; micturition; retching; stimulation of mucous glands; dyspnea; muscular weakness and collapse; with anxious expression of face.

DOGS:

Excitement; swallowing movement; retching and vomiting; muscular weakness; involuntary defecations (diarrhea).

SHEEP:

Labored, rapid respirations; mucous discharge from nose; tears; frothing at the mouth; swallowing movements; cyanosis of skin and mucous membranes; violent trembling; protrusion of tongue; collapse.

HOGS:

Restlessness and excitement; labored, stertorous breathing; cyanosis, very marked in white hogs; muscular weakness and collapse; vomiting (occasionally); involuntary defecation.

RABBITS:

Slow respirations, but absence of dyspnea; weakness, followed by falling on one side and remaining motionless; involuntary defecation; increase of peristalsis; dilatation of the pupils; pallor of the visible mucosæ; convulsions; absence of heart-beat and arrested respiration.

GUINEA-PIGS:

Restlessness; sneezing and rubbing of nose; muscular weakness, followed by recumbency; convulsions; slow, labored respirations; slow but strong, regular heart-beats; dilatation of the pupils; involuntary micturition and defecation; cyanosis.

MAN:

Fever; skin eruptions (rash or urticaria); swelling of the lymph-glands; edema; leukopenia, shown by blood counts; pains in the joints.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 215, 1919.

(Reprinted from *The Journal of Biological Chemistry,* Vol. XXXVIII, No. 3, 1919.)

THE ABSENCE OF FAT-SOLUBLE A VITAMIN IN CERTAIN DUCTLESS GLANDS.*

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Of late a good deal of emphasis has been placed upon the possible value of the fat-soluble A accessory in the treatment of certain diseases such as rickets, pellagra, and xerophthalmia. It is known for example that some of the oils, such as cod liver oil, which are admitted to be of therapeutic value, contain a vitamin or food hormone. In 1909 Stepp (1) concluded from his experimental studies on rats that certain fats or related substances, such as lipins, which are soluble in fat solvents, were essential for maintenance and growth. McCollum and Davis (2) and Osborne and Mendel (3) came to the same conclusion in 1913 when they discovered that butter fat stimulated growth in young rats that had thrived normally for a long time and then had ceased to grow. Osborne and Mendel (4) found that this accessory resided in the butter oil fraction and had no relation to lipoids; that it was soluble in absolute alcohol, and when introduced into the diet cured an eye trouble which later McCollum classed as a form of xerophthalmia (5). McCollum and associates who designated this accessory as fat-soluble A (6) found: that, if it was present in animal tissues, it was removed with the fats when they were dissolved out by ordinary solvents such as ether; but in vegetable tissues (plants, seeds, or leaves) it remained behind when the oils were extracted with ether, benzine, chloroform, or acetone (5, 7). Hot alcohol, however, removed it from the residue (7, 8).

The following table gives a current summary of the presence or

*Read before the Buffalo meeting, American Chemical Society, April 9, 1919.

absence of the fat-soluble A vitamine in food substances, based upon published results.

Present.

(*a*) Animal Source:
- Butter fat (2, 3).
- Egg yolk and fat (2, 4, 9).
- Cod liver oil (9).
- Beef oil (10).
- Oleomargarine (10, 11).
- Cod testicles (12).
- Pig kidneys (12, 13, 14).
- Pig liver and liver oil (14).
- Fat fish and fish oils (15).
- Dried and unsweetened condensed milk (16).

(*b*) Vegetable Source:
- Corn, wheat germ, rye, and oats (13).
- Leaves of plants (5, 7, 17, 18).
- Cotton seed flour and oil (19).
- Flaxseed, millet, and hemp seed (7).
- Soy beans (20).
- Peas (21, 22).
- Bananas (23).

Absent or very little.

(*a*) Animal Source:
- Lard (2, 4).
- Pig heart (13, 14).

(*b*) Vegetable Source:
- Olive oil (2).
- Almond oil (9).
- Oils from maize, linseed, sunflower, soy beans, and wheat (17).
- Cotton seed oil (12, 17).
- Nut margarine (11).
- Vegetable margarine (11).
- White beans (24).
- Barley (25).
- Potato (26).

From the above list, it will be seen that one of the ductless glands, the cod testicles, has been found to contain the fat-soluble A in the ether extract. In this report we add our findings, made upon the fats from three other ductless glands—pancreas, thymus, and suprarenal.

The glands were ground and extracted with either acetone or benzine. The fats were redissolved in ether and again filtered. They were then incorporated in a diet that had been found to be adequate for growth except for a lack of the fat-soluble A accessory. The deficient diet was first fed to rats in order to bring them down to a plane where they would show definite symptoms of a lack of this vitamin, such as loss in weight, poor physical condition, and xerophthalmia. The rats were then fed the modified diet and the results compared with control tests (Chart 1), carried out under the same conditions. Ten per cent of the glandular fat was used.

The weight curves, given in Charts 2, 3, 4, and 5, show definitely that benzine or acetone did not extract from the pancreas, thymus, and suprarenal glands a fat that contained the fat-soluble

A vitamin. Therefore, in the use of desiccated products of these glands, it is safe to state that any of the ether-soluble fat which is still present (the major portion being removed before desiccation) does not have any therapeutic value from the standpoint of the fat-soluble A vitamin.

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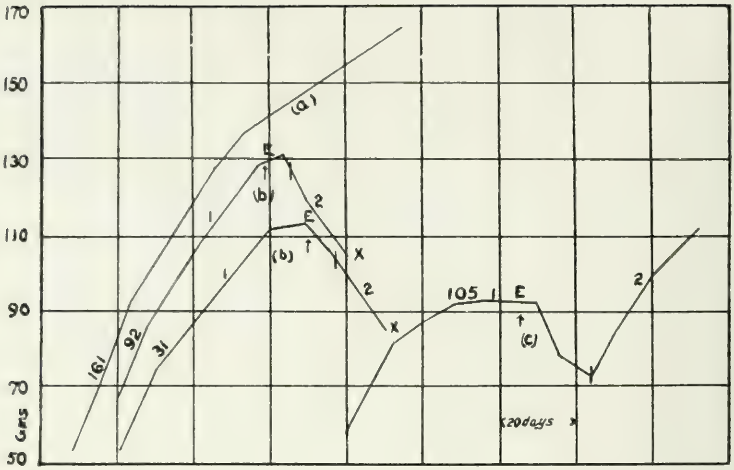

CHART 1. Butter fat. The curves in this chart represent the control groups; (*a*) rats on a diet with fat-soluble A present, (*b*) rats on a diet with fat-soluble A absent, and (*c*) rats that were first put on a deficient fat-soluble A diet, Period 1, and then cured by introducing this accessory into the food mixture, Period 2. "E" designates when xerophthalmia became evident. The basal diet was made up of lactalbumin protein 10 per cent; lard 28 per cent; protein-free milk (the carrier of the water-soluble B vitamin, mineral salts, and lactose) 28 per cent; and starch. Ten per cent of purified butter fat was employed to supply the fat-soluble A and replaced an equivalent amount of lard.

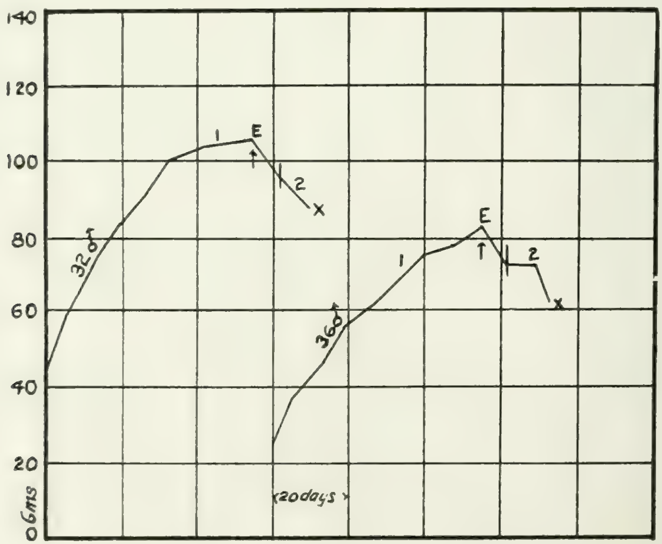

CHART 2. Pancreas fat. In Period 1, the rats were fed the basal diet. Period 2, when the acetone-extracted pancreas fat was used, indicates that the rats did not respond to the change in diet but died as designated by "X."

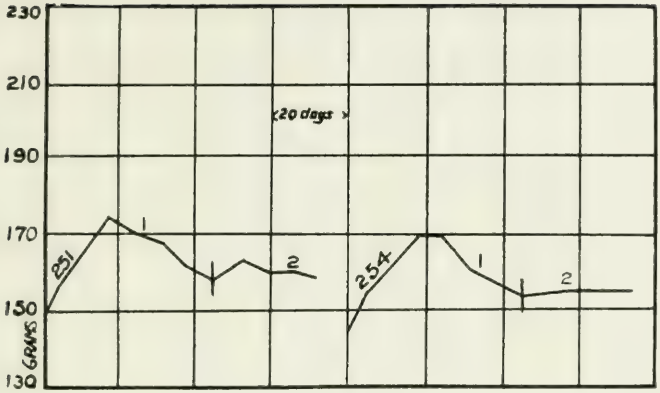

CHART 3. Pancreas fat. In Period 1, the basal diet was used. In Period 2, 10 per cent of the pancreas fat was introduced into the ration. It was obtained by extracting the glands with ether instead of acetone as in Chart 2. These rats were much heavier at the start than those represented in Chart 2. There was a cessation in the loss of weight, but no gain in weight and no improvement in bodily condition. The rats finally became so weak that they were killed.

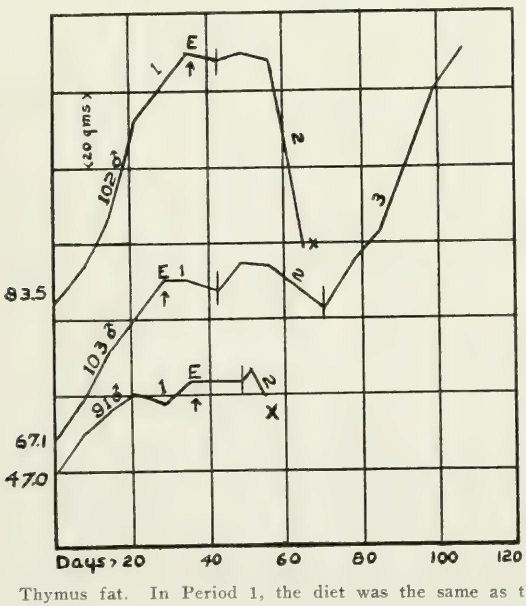

CHART 4. Thymus fat. In Period 1, the diet was the same as the basal food fed the rats represented in Charts 1, 2, and 3. Period 2 shows when the acetone-extracted thymus fat was introduced; there was no beneficial effect resulting, only a continued decline. In Period 3, the equivalent amount of butter fat, substituted for the thymus fat, brought about immediate response.

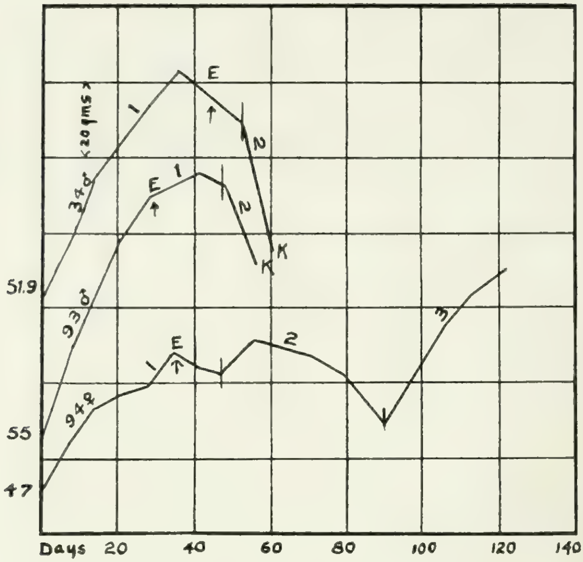

CHART 5. Suprarenal fat. At the end of Period 1, during which the basal diet was fed, 10 per cent of the ether-extracted suprarenal fat was put in the food mixture, in place of so much lard. No growth, gain in weight, or improvement in body took place. Upon introducing 10 per cent of purified butter fat for the suprarenal fat, an immediate response in condition and weight was manifested. Rats 34 and 93 were in such an emaciated condition that they were killed, as shown in the chart by "K."

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 216, 1919

(Reprint from *Surgery, Gynecology and Obstetrics*, July, 1919, pages 92-94.)

ADRENALIN.

AN ADJUNCT TO AND AN ANTIDOTE FOR APOTHESINE.

By Herbert C. Hamilton, Detroit, Michigan.

Almost every medicinal substance is a potential poison and should be considered from this viewpoint. When for any reason toxic symptoms follow its administration, the physiological effects of a toxic dose should be known and information available as to a counteracting substance.

Under certain conditions local anesthetics may produce secondary effects of an entirely different nature from their beneficial anesthetic properties. In the case of cocaine the high degree of toxicity and its habit-forming effects are such as to limit or inhibit its use in many cases.

Among the substitutes for cocaine, using the term in the sense of an equivalent rather than an inferior substance, novocaine for some years became very popular. Braun (1) states: "Experience and experiment have shown that by doubling the dose of novocaine so as to make it as effective as cocaine and at the same time by adding suprarenin (adrenalin), novocaine has become the ideal anesthetic for injecting into the tissues and has made the use of cocaine unnecessary."

Attempts have been made in some cases to overcome certain of the objectionable features of cocaine by a combination of chemicals which will enhance its value, either (1) directly as by applying the cocaine in its basic form, (2) or indirectly by localizing its action and so making a smaller dose more effective. Braun (3) says: "It was observed that the local anesthetic power of cocaine was enormously increased by the addition of very small quantities of suprarenin."

Apothesine is a local anesthetic which is materially less toxic

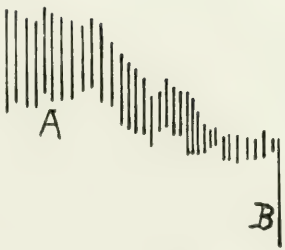

Fig. 1.

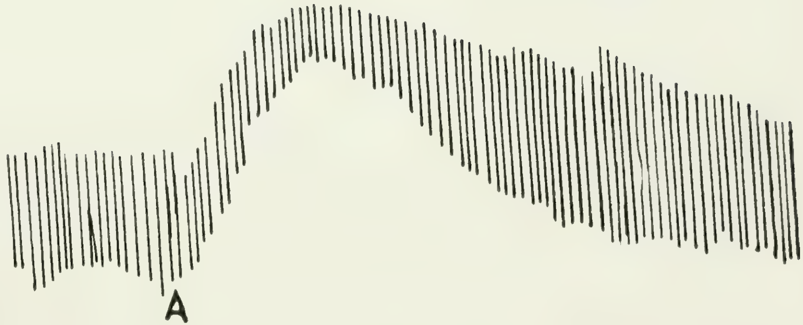

Fig. 2.

Fig. 1. *A*, Injection of fatal intravenous dose of apothesine. *B*, Death of dog.
Fig. 2. Injection of 0.00001 gram adrenalin as solution of adrenalin chloride at *A*.

than cocaine and is entirely free from habit-forming effects. By laboratory tests, approximating two of its methods of application—on the sciatic nerve of the frog and intracutaneously applied to human subjects—it is demonstrated to be equal to cocaine; in its action on mucous membrane, while less effective than cocaine, it is identical in degree and kind with that of novocaine (4). Its toxic action is also identical with that of novocaine, showing a strongly depressing effect on the heart and respiration.

While local anesthetics are ordinarily used in doses minute compared to the amount necessary to produce a systemic reaction, there are occasions in which such reactions are evident either because of an excessive dose, or because of hypersensitiveness to its toxic action. In such cases knowledge of an antidote or counteracting substance is of primary importance, and should be in the hands of every user of the anesthetic.

The minimum fatal subcutaneous dose of apothesine, based on guinea-pig experimentation, is several times that for cocaine, that is, it is much less toxic. The fatal dose calculated to the average weight of a man is more than a half ounce of the pure crystals.

The toxic effects are exerted largely on the circulatory system. Blood pressure and heart amplitude are greatly reduced under its influence in animal experiments and death is apparently due to the depression of the heart.

What is therefore more natural than to turn to adrenalin, the characteristic effects of which on the circulatory system are exactly the opposite of those of apothesine and which when properly administered acts even more quickly. Experiments on anesthetized dogs confirmed this theoretical counteracting effect and proved, as shown by the illustrations, that an otherwise fatal dose could be favorably influenced by administering adrenalin chloride solution, and that when the latter was injected with the apothesine intravenously, its depressing action on the heart could be eliminated entirely.

Although in rare cases only is apothesine injected intravenously, its effect from this form of administration is much more rapid and is identical in kind with that from the subcutaneous injection, and is produced by a much smaller dose. The results, therefore, are comparable, although the doses are not.

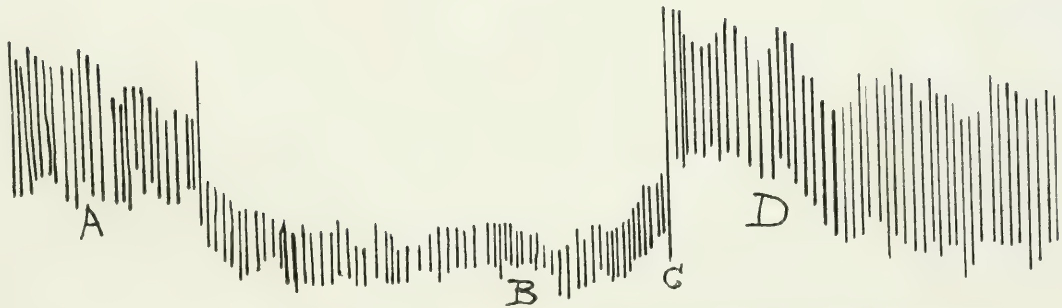

Fig. 3. *A*, Injection of 0.08 gram apothesine intravenously. *B*, Injection of 0.00001 gram adrenalin. *C*, After complete recovery, injection of 0.08 gram apothesine and 0.00001 gram adrenalin. Note that at D the fall in pressure is promptly neutralized by the pressor effect of the adrenalin.

The experiments were carried out on anesthetized dogs, the apothesine being administered intravenously on account of the promptness of its action when so given. The effect on the heart when so administered is so rapid that it was found necessary to mix the two substances, since the one acts almost as promptly as the other. When the two agents are injected at the same time, the depressing effect of a large dose of apothesine is not evident until the action of the adrenalin has ceased to be a factor in sustaining the amplitude of the heart beats and the blood pressure. When this occurs, due to an insufficient dose of adrenalin, a second small dose will restore the circulation to its former condition and sustain blood pressure until the toxic effects of the apothesine are gone, and the dog will remain in good condition.

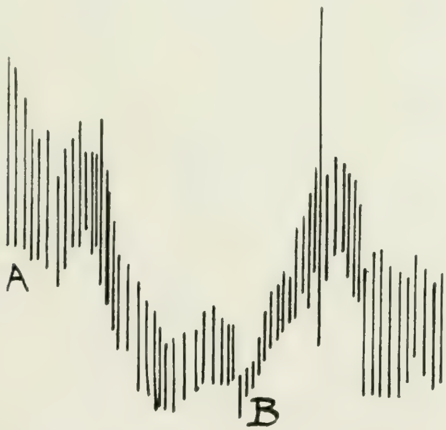

Fig. 4. *A*, Injection of 0.32 gram apothesine and 0.00001 gram adrenalin. *B*, Injection of 0.00001 gram adrenalin.

The tracings shown are those typical of apothesine, of adrenalin, and of the resulting effects from judicious mixtures of the two: The dose of adrenalin which raises the blood pressure of a dog is 0.00001 gram or 1 cc of adrenalin chloride 1:100,000, a dose varying not so much with the weight of the dog as with the condition of the circulatory system. A dose ten times as great has a much more pronounced effect but not serious. From a mixture of the two intravenously administered and with a subsequent dose of adrenalin if necessary, blood pressure and heart action can be

so controlled that no serious results follow the administration of a dose of apothesine which without the adrenalin would have been fatal within a short time.

The simultaneous administration of adrenalin with apothesine, as with cocaine and novocaine, is not for any purpose other than to localize and intensify the anesthetic action, since its stimulating action on the heart and vessels is obtained promptly only by intravenous injection. But the antidotal effect is indirectly effective even from the subcutaneous administration because adrenalin prevents general absorption of the anesthetic and by intensifying the action of the apothesine makes large doses unnecessary.

The value of adrenalin, therefore, for both purposes, to limit absorption and to sustain the heart, is due to its characteristic properties of constricting the blood-vessels and acting as a prompt and powerful cardiac tonic.

NOTE.—At the request of the publishers the original tracings are replaced by line drawings to obtain better reproductions.

REFERENCES.

[1]Braun. Shield's Translation, p. 123.
[2]Gros. Arch. f. Path. u. Pharmakol., 1910, lxiii, 80; 1912, lxv, 127.
[3]Braun. p. 139.
[4]Hamilton. J. Lab. & Clin. Med., 1918, iv, 60.

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 217, 1919

(Reprinted from *The Northwestern Druggist*, July, 1919.)

CRAMP BARK, HIGHBUSH CRANBERRY.

An Interesting Explanation on the Substitution of Maple Bark for Viburnum.

By Oliver A. Farwell, Detroit, Mich.

Since it was shown in 1913 that the commercial bark, known as Cramp Bark, was derived from *Acer spicatum* Lam., there has been considerable discussion as to when the substitution of this bark for that of the *Viburnum Americanum* Mill. actually took place, and to which of these species the name Cramp Bark should be restricted. In my paper published in the *Bulletin of Pharmacy* in 1913, I showed that Maple Bark was the commercial Cramp Bark of the early nineties and that it was this Maple Bark that was described in the U. S. P. VII. It is certain that Maple Bark was being collected as Cramp Bark for at least 20 years prior to that date for commercial manufacturing purposes. It has been claimed that true Viburnum Opulus was collected and used by the early eclectics, that is, from *Viburnum Americanum* Mill. But on the other hand, it seems most probable to me, from the evidence I have been able to gather, that the bark gathered in large quantities for the commercial drug markets always has been Maple Bark; consequently, as no Viburnum Americanum bark was ever gathered commercially for commercial purposes, no substitution ever took place. The use of Maple Bark as Cramp Bark was the result of a mistaken identification by some one right at the start of its commercial history. Probably both manufacturers and collectors were guided by a more or less imperfect description of the bark desired and without authentic samples for comparison. Externally the barks of Mountain Maple and of Highbush Cranberry bear a strong resemblance one to the other. Hence, the Maple being far more plentiful and answering very well to the description in hand, it was gathered and accepted from the very beginning by all concerned and without question as to the true Cramp Bark. This condition was continued without question down to 1913. The name, of course, originated in Europe where it was adopted for the bark of the European *Viburnum*

Opulus Lin. because it was used in the treatment of cramps. Whether or not the bark of the European shrub was ever imported I am unable to say. In 1912, the only Cramp Bark on the European drug market was of American origin and mostly Maple Bark, but in some instances it was Black Haw, *Viburnum Prunifolium* Linn. *Viburnum Americanum* Mill. is known throughout its region of growth most generally under the name of Highbush Cranberry and has been marketed under this name. The name "Cramp Bark," I believe, the Federal Pure Food and Drug authorities have ruled should be considered as applying to the bark of the *Viburnum Opulus.* A custom of 50 years standing will be difficult to eradicate, and for a long time to come the use of the name Cramp Bark in the commercial world, as consequence of the above ruling, will lead to a large amount of confusion and probably of financial loss to many; in view of this fact, I feel emboldened to express my individual opinion that there would have been less confusion and that all interests would have been equally well conserved if the name Cramp Bark should have been retained for *Acer spicatum* Lam. and if the name Highbush Cranberry should have been adopted for the *Viburnum Americanum* Mill. Use determines the application of a name, and 50 years should be sufficient to determine its permanent application. Most names sooner or later lose the original meaning appertaining to them. *Tempora mutantur, et nos mutamur in illis.*

I wish to thank Mr. John Uri Lloyd for hearty encouragement and for samples of Viburnum Americanum bark representing a drug used by his establishment in the early seventies. I have been unable to obtain samples of drug used in the sixties.

A letter from Mr. Lloyd bearing upon the subject I am sure will be of interest to all, and I herewith print it with his permission.

Cincinnati, Jan. 29, 1919.

Dr. Oliver A. Farwell,
c/o Parke, Davis & Co.,
Detroit, Mich.

My dear Dr. Farwell:—

In reply to your letter of Jan. 22nd I will say that the burning of our laboratory, about twenty-five years ago, destroyed most of my specimens accumulated to that date. I was in hopes, however, that I could put my hand on a specimen of Cramp Bark

gathered in the early day, before the introduction of the proprietary medicines which made the demand for Cramp Bark in the American drug market, and in my opinion led to the introduction of Maple Bark. Let us not, however, neglect that possibly both barks were once used under the name "Cramp Bark," although I have no record of Maple Bark ever having been given that common name, or used in domestic medicine in the field Viburnum opulus occupies.

It may interest you to have from me a description of the methods that an establishment like ours employed, fifty and sixty years ago, to obtain their crude materials. At that date Cincinnati was the headquarters for botanical drugs, it being the headquarters for the Eclectic school in medicine, the members of which give their particular attention to botanical drugs.

Such men as Dr. John King, by correspondence with physicians over the country, learned the locations of the drugs they needed, and employed physicians to oversee their collection. These were unquestionably all true to name, for both the physician and such men as Dr. King, who used the drugs in making his home-made samples, were very careful regarding the authenticity of the crude material.

In this connection I will state that preceding the day I have mentioned such enthusiastic herbalists as Samuel Thomson traveled the country over to collect drugs in their natural locations. You will find in the narrative of his life, Thomson refers to the fact that he traveled to a distant part of New England for the purpose of collecting Ceanothus Americana, or as he called it, "Red root," which was not found in his New England neighborhood.

Viburnum opulus, under the name Cramp Bark, was the only Virburnum used in medicine when I entered the field, and I will add the only Cramp Bark used in medicine at that date. It was a favorite of Dr. King and others, the bark that came into the hands of our establishment being gathered in New England, under the direction of physicians who supplied it, not for profit, but as an accommodation to the profession at large, Viburnum opulus not being native to our section of the country. It may interest you to know that preceding your admirable paper showing that the Maple was used as Cramp Bark, I was aware that something had been substituted, different from the old Cramp Bark and at that very date was paying \$1.25 a pound for Viburnum opulus when

the market was flooded with Cramp Bark under the name "Cramp Bark" at 12c a pound. Not one word did I say concerning the subject, because in the multiplicity of my duties I did not reach that problem, nor have I said anything since regarding the excessive price we were forced to pay to obtain the true Cramp Bark, which we obtained in the old-fashioned method, from collectors in whom we had confidence. I am sending you herewith a few samples of the drug under consideration, none of which, however, antedated 1870.

Be it known that the great demand for Cramp Bark in the market arose after Viburnum Cordial and proprietary compounds were advertised to the profession, as well as to the people. In my opinion it would have been practically impossible, at those dates, at any reasonable price, to have obtained enough Virburnum opulus to have supplied these specialists' demands, and in my opinion the specialists themselves, as well perhaps as the collectors, in good faith accepted that the material used by them was the Cramp Bark of old.

Possibly my remarks have not been discursively exact enough for your purpose, but at least I believe you can catch from what I have said that in the early date there was no question about the authenticity of Cramp Bark, which was surely Viburnum opulus. In conclusion I will state that I have not yet given up my search for a specimen of our Cramp Bark preceding 1870, and shall not, because occasionally the unexpected happens, from unlooked for sources. From this you will perceive that in my opinion you are absolutely correct in your surmise regarding the manner in which Acer displaced Viburnum, under the name Cramp Bark, but again I would like to ask you to bear in mind that possibly Acer was somewhere known as Cramp Bark, and used under the name Cramp Bark, unbeknown to professional print.

And now I will thank you for the reprints you have sent me on Brazilian Jalap and other botanical problems. These I shall read with great care, and thank you herewith for the opportunity, as well as congratulate us all on the interest you are taking in this direction.

With my kindest regard and best wishes, I am,

Sincerely yours,

(Signed) John Uri Lloyd.

**Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 218, 1919.**

(Reprinted from the *American Midland Naturalist,* Vol. VI, Nos. 3, 4, May-July, 1919.)

PANICUM LINEARE, LINN.

BY OLIVER A. FARWELL.

(Department of Botany, Parke, Davis & Co., Detroit, Mich.)

In the Species Plantarum Ed. 2, Vol. 1, p. 85, 1762, Linnaeus published *Panicum lineare* as a new species with the following description: "Panicum spicis digitatis subquaternis linearibus, flosculis solitariis secundis muticis. Habitat in Indiis. Culmi prostrati, laeves, ramosi. Spiculae lineares, rectae, angustae. Flores subtus alterni. Calycis squama exterior brevior, patens, rachi adherens."

There is no specimen in the Linnaean Herbarium and there is no reference to older authors. The species must be interpreted from the description alone. It is self-evident from the diagnosis that the species belongs to the Digitaria group. It can not be *P. Dactylon* Linn. since it is *laeves* and *prostrate,* while the latter has the spikes *basi interiore villosis* and the plant is *sarmentis repentibus; it* cannot be *P. sanguinale* Linn. or *P. filiforme* Linn. since in these the flowers are *in pairs* while in *P. lineare* they are placed *singly*. The habitat *in Indiis* may mean India and the East Indies, or it may mean in both the East and West Indies. Linnaeus used it both ways, I believe, but in the present instance the presumption is that he meant in both the East and West Indies since in the Mantissa II 323, 1771, he refers to his *P. lineare,* without comment, the *P. lineare* Burm. Ind. 25, t. 10, f. 2, and the *Gramen Dactylon,* etc., Sloane, hist. 1, p. 113, t. 70, f. 3. We can not, therefore, neglect a study of the West Indian species in endeavoring to ascertain the proper application of the Linnaean name. Mr. Hitchcock in Contributions U.S. Nat. Herb. XII, pp. 134, 142 and 209, 1908-9, has shown that the Sloane reference belongs to what is now known as *Syntherisma setosa* (Desv.) Nash or *S. digitata* (Swz.) A. S. Hitch.; he combines under the latter name two forms that have been variously considered as distinct species

or as varieties of *Panicum sanguinale* Linn. Grisebach, Flora Br. W. Ind. 544, 1864, has described these West Indian forms as *pilose;* therefore, they can scarcely be considered as belonging to *P. lineare* Linn. which was described as *laeves*. It has been customary to accept Burmann's *Panicum lineare* Fl. Ind. 25, pl. 10, fig. 2 (fig. 3 of the plate) as typifying the Linnaean species, but this cannot be adhered to as it did not make its appearance until 6 years after Linnaeus had published his *P. lineare*. Burmann uses the Linnaean diagnostic description verbatim and the references to Sloane mentioned above, with the added remark: "Specimina ex India missa variant spicis saepe binis linaeribus and flosculis alternis." Since Linnaeus assisted Burmann in the preparation of his Flora Indica, the above phrase seems to indicate the former thought that the variable specimens from India were not typical of his *P. lineare* but could be referred to it; also it may be considered as more evidence that the original habitat "in Indiis" as given by Linnaeus did not include India but referred more especially to the East and West Indian Archipelagoes. It seems probable that fig. 2 (which is fig. 3 of the plate due to a transposition of the numbers) represents the variable plants of India mentioned in the supplementary paragraph. Hooker, Flora Br. India VII, 289, 1897, refers Burmann's *P. lineare* to *Cynodon Dactylon* (Linn.) Pers. I have seen specimens of the latter from India that might be said to be fairly well represented by Burmann's figure if it may be viewed in the light of a *very crude drawing*. The Index Kewensis, Vol. II, 415, 1895, refers it to *Paspalum brevifolium* Fluegge; but Hooker, l. c., 18, describes this species as with *geminate* flowers, one sessile and the other pedicelled, which is at wide variance with Burmann's figure, and the Linnaean description. The Index Kewensis recognizes *Panicum glabrum* Gaud. as a valid species; also *P. lineare* Linn. as a valid species of N. America, but I am unable to interpret it.

The Linnaean description as compared with modern day descriptions is rather incomplete; in so far as it goes, however, it is characteristic of the plant that has generally been known as *Panicum glabrum* Gaud. Likewise, by the process of eliminating all related species which it cannot possibly be, we gradually come to the same species, *Panicum glabrum* Gaud. The last sentence of the Linnaean description referring to the exterior glume spread-

ing and adhering to the rachis is not always apparent, but I have seen just such a condition in some specimens of *Digitaria humifusa* collected in Michigan. This species is not usually accredited to the "Indies" in botanical manuals, but Hooker, l. c., 17, gives it for the Himalaya Mts. and as far south as Simla, and the Index Kewensis to North Temperate and Tropical regions. Perhaps Linnaeus was misinformed as to its distribution, and as happened in other cases with him, gave accordingly a wrong habitat. The more important synonyms are given below.

Digitaria linearis (Linn.) Pers. Syn. 1, 85, 1805; Crep. Man. ed. 2, 335, 1866. *Panicum linearis* Linn. Sp. Pl. Ed. 2, Vol. 1, 85, 1762; Burm. Fl. Ind. 25 pl. 10 fig. 2 (3), 1768; Krock. Fl. Siles. 1, 95, 1787. *Syntherisma linearis* (Linn.) Nash, Bull. Torr. Bot. Club, XXII, 420, 1895. *Panicum Ischaemum* Schreb. ex. Schweigg. Spec. Fl. Erlang, I, 16, 1804. *Digitaria humifusa* Pers., l. c. *Paspalum ambiguum* Lam. and D. C., Fl. Fr. III, 16, 1805. *Syntherisma glabrum* Schrad. Fl. Germ. 163, t. 3, fig. 7, 1806.

I wish to thank Dr. Nieuwland of Notre Dame for courtesies shown me in connection with this study.

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 219, 1919

(Reprint from *Journal of American Veterinary Medical Association,* Vol. LV, New Series, Vol. 8, No. 4, July, 1919, pp. 394-401.)

BLACKLEG AGGRESSIN.*

HERBERT C. WARD.

(From the Medical Research Laboratories of Parke, Davis & Co., Detroit, Michigan.)

For more than a century men have known something about a disease called Blackleg. Over forty years ago the specific agent was discovered, and for thirty years cattle have been successfully protected against this infectious plague. Before practical methods of immunity were discovered tremendous losses, ranging from 5% to 25%, occurred among the young stock in certain areas of world-wide distribution. Vaccines reduced these losses to 1% and even less. But to the stockowner as certainly as to the public welfare the loss of even 1% of such live stock wealth is still a most serious economic burden. Therefore we understand why men have been stimulated to improve their methods in the manufacture of an ideal vaccine. The crude vaccines of the past have rendered historical service, but today we have hope of greater efficiency in a new and better method.

Attenuated vaccines were made both from diseased tissues and from pure cultures. Their protective values were determined upon tens of thousands of cattle in the afflicted districts, as well as upon experimental animals of the laboratory. Naturally, the most successful vaccines became more and more extensively employed. But failure following applications in their use were continually suggesting the need of an improved and safer protection. Vaccines were subject, therefore, to new processes of attenuation. Animals were injected in different portions of the body, and efforts were made to improve these immunizing substances.

This effort to secure a more satisfactory vaccine has brought us to the present consideration of a group of blackleg immunizing products known as aggressins.

*Paper read before the Southeastern Michigan Veterinary Medical Association.

Inquiry as to the exact meaning of aggressin informs one that this term has been used to express a theoretical course of immunity reaction when a bacterial exudate is injected into the living body. Bail reported that exudates from infective processes produced an active immunity against the original excitant of the specific disease. His further observations led him to report that the leukocytes were inactive, being destroyed or held back by some potent force in the exudate; so that in the absence of these cells the blackleg or other bacilli were able to rapidly multiply and complete for that animal the course of a natural infection. According to his opinion, then, this potent force in bacterial exudate is active, offensive, destroying somewhat the resident defenses of the body. This action is due to some toxic substance produced by the blackleg organism as it develops in the tissues during infection. Because these exudates possess this aggressive power they have been popularly termed aggressins.

Let us take, therefore, a small amount of the exudate from the muscle of a bovine dead of blackleg in the field and inject this into a healthy animal. What happens? The aggressin clears the way for a free, unrestrained development of the blackleg organism and the animal sinks before the onslaught of an acute infection.

When we remove the living virus, filtering out the spores and bacilli from this exudate, no such infection develops, no local or general reaction occurs. Therefore if toxic principles are produced, they exist only in physiological proportions. Exposing a treated animal later to blackleg virus no infection follows in the presence of an active immunity. For a short and rather indefinite period following treatment with this germ-free exudate, or aggressin, calves become highly sensitive to blackleg infection, and if exposed to a natural infection or to experimental virus will die as though untreated. This hypersensitive condition passes away in a few days, and it is then practically impossible to produce blackleg with even a double dose of experimental virus.

Repeated demonstration of the practical truth of these results underlies our success in immunizing susceptible animals with blackleg aggressin.

Aggressins were first used experimentally on laboratory animals by Bail and were obtained by pipetting off the peritoneal

exudate from his infected animals. Schobl, acting on Bail's suggestive work, first experimented with blackleg aggressin on guinea pigs. To secure his exudates he injected guinea pigs subcutaneously and at the climax of the infection withdrew the tissue juice, centrifuged it, and shook with toluol. The clear liquid was then tested free of bacteria. This immunized guinea pigs against a virus successfully after a ten-day period.

For large amounts of aggressin he injected a calf with blackleg tissue virus and obtained the edematous extract. This was then centrifuged clear and preserved with toluol. After filtration through Berkefeld it was proved to be germ-free by both cultural and animal tests.

More recently R. A. Kelser reported his method of manufacturing blackleg aggressin. Animals received muscle virus intramuscularly, and following death the edematous fluid and affected muscle tissues were collected, mixed, and ground up. This mixture was then frozen and left for a few hours, after which thawing was facilitated and the filtrate obtained by pressing and filtering. His product was found to possess the same immunizing properties as discovered by Schobl.

The commercial processes of manufacturing wholesale amounts of blackleg aggressin vary only in mechanical details. Susceptible animals are subjected to fatal artificial infection with muscle virus or cultures. A few hours after death the hair and hide are removed, the edematous juices collected, and the affected muscle tissues excised and pressed until all the liquid has been obtained. This is filtered and preserved until free of all viable cells and spores. After double testing by sterility and safety trials this filtrate is delivered to the market as germ-free blackleg aggressin.

When we seek for a demonstration of the protective value of blackleg aggressin it can be found in the report of Schobl, who used successfully both guinea pigs and calves. His results are quoted in the following Table No. 1.

Table No. 1.

AGGRESSIN IMMUNITY TESTS BY O. SCHOBL.

Animal Groups	*Aggressin*	*Time Before*	*Infection With*	*Results*	
Guinea Pigs No. 1	0.5 mil.	0	B. S.		Died
2	0.5 mil.	0	"		"
" 1	1.0 mil.	2 days	Exudate		Died
2	1.0 mil.	"	"		"
3	0	"	"		"
4	0	"	"		"
" 1	1.0 mil.	4 days	Exudate	Lived	
2	1.0 mil.	"	"	"	
3	0	"	"		Died
4	0	"	"		"
" 1	0.5 mil.	6 days	Meat	Lived	
2	0.5 mil.	"	"	"	
3	0.5 mil.	"	"	"	
4	0	"	"		Died
5	0	"	"		"
" 1	0.5 mil.	10 days	Exudate	Lived	
2	0.5 mil.	"	"	"	
3	0	"	"		Died
4	0	"	"		"
" 1	0.5 mil.	21 days	Meat	Lived	
2	0.5 mil.	"	"	"	
3	0	"	"		Died
4	0	"	"		"
" 1	1.5 mil.	51 days	Meat	Lived	
2	0	"	"		Died
3	0	"	"		"
Heifers No. 4	5.0 mil.	10 weeks	Meat Virus	Lived	
5	7.0 mil.	2 weeks	"	"	
6	0		"		*

*Died from acute infection.

His study showed that animals were not protected immediately following aggressin injection. After three days a developmental immunity was established and remained active as long as the tests were continued.

Kelser failed to demonstrate satisfactorily immunity values when he used guinea pigs, but his results with calves were excellent, and are quoted in the following table.

TABLE NO. 2.

Animal	Aggressin	Time Before	Infection With	Results	
Calf No. 1	5 mils	14 days	Muscle virus	Lived	.....
" 2	5 mils	"	"	"	.....
" 3	0	"	"	.....	Died
" 4	0	"	"	.....	"
" 5	0	"	"	.....	"
" 6	0	"	"	.....	"
" 1	5 mils	$5\frac{1}{2}$ months	Muscle virus	Lived	.....
" 2	5 mils	"	"	"	.....
" 3	0	"	"	.....	Died
" 4	0	"	"	.....	"
" 5	0	"	"	.....	"
" 6	0	"	"	.....	"

These findings indicate perfect protection and permanency of the acquired immunity. It is important to call attention to the fact that the treated animals were not infected until after a period of fourteen days.

With the suggestive results already reported in mind, we applied the same kind of tests upon blackleg aggressin as it is being placed on the market today. The following tables represent the results of our experimental studies.

A few animals died from mixed infections and in all such sets* duplicate trials were made with satisfactory results. Careful checks were made of every animal dying under tests. The results (Table 3) constitute, therefore, a demonstration of the protective value of blackleg aggressin against blackleg experimentally produced in guinea pigs. This protection did not appear to have developed until the third day.

Before proceeding to the test on bovines it was necessary to gauge as near as possible the cultural virus and in order to demonstrate viable pathogens in the culture virus recourse was had to subjecting guinea pigs to graded doses. The results of such a titration are to be seen in Table 4. All animals were posted and the purity of the virus established microscopically and culturally.

Both strains of the blackleg virus were active, therefore, in doses of 0.1 mil or less for guinea pigs.

Table No. 3.

Animal	*Dosage*	*Time Before*	*Infection With*	*Results*	
Guinea Pig 1	1. mil.	1 day	Muscle virus No. 4	Lived	.....
2	1. mil.	"	"	.....	Died
3	Control	"	"	.....	"
" 1	1 mil.	2 days	"	Lived	.....
2	1 mil.	"	"	.....	Died
3	Control	"	"	.....	"
" 1	1 mil.	3 days	"	Lived	.....
2	1 mil.	"	"	"	.....
3	Control	"	"	.....	Died
" 1	1 mil.	4 days	"	Lived	.....
2	1 mil.	"	"	"	.....
3	Control	"	"	.....	Died
" 1	1 mil.	6 days	"	Lived	.....
2	1 mil.	"	"	"	.....
3	Control	"	"	.....	Died
" 1	1 mil.	7 days	"	Lived	.....
2*	1 mil.	"	"	"	.....
3	Control	"	"	.....	Died
" 1	1 mil.	8 days	"	Lived	.....
2*	1 mil.	"	"	"	.....
3	Control	"	"	.....	Died
" 1	1 mil.	10 days	"	Lived	.....
2	1 mil.	"	"	.....	Died
3	Control	"	"	.....	"
" 1*	1 mil.	12 days	M. virus also culture	Lived	.....
2*	1 mil.	"	"	"	.....
3	Control	"	"	.....	Died

Table No. 4.

VIRUS No. 7 TITRATION

Animal No. 1	Rec. Subc.	1.0 mil.	Virus No. 7	Dead in 18 hours
Animal No. 2	Rec. Subc.	0.5 mil.	Virus No. 7	Dead in 18 hours
Animal No. 3	Rec. Subc.	0.2 mil.	Virus No. 7	Dead in 18 hours
Animal No. 4	Rec. Subc.	0.1 mil.	Virus No. 7	Dead in 18 hours

VIRUS No. 3 TITRATION

Animal No. 1	Rec. Subc.	1.0 mil.	Virus No. 3	Dead in 16 hours
Animal No. 2	Rec. Subc.	0.5 mil.	Virus No. 3	Dead in 16 hours
Animal No. 3	Rec. Subc.	0.1 mil.	Virus No. 3	Dead in 32 hours
Animal No. 4	Rec. Subc.	0.05 mil.	Virus No. 3	Dead in 38 hours
Animal No. 5	Rec. Subc.	0.01 mil.	Virus No. 3	Sick (?)
Animal No. 6	Rec. Subc.	0.01 mil.	Virus No. 3	Sick (?)

Such a titration was used as a guide by which the dosage for bovines could be determined. The final dosage was set at 5 mils and used as stated in the following tables. Virus No. 7 was used on the first three heifers, Table No. 5, and Virus No. 3 on the last three, Table No. 6.

TABLE No. 5.

AGGRESSIN IMMUNITY TESTS ON BOVINES.

Heifer	*Immunized*	*Method*	*Aggressin*	*Inoculated*	*Method*	*Virus*	*Result*
No. 1....	2-5-19	Subc.	10.0	2-19-19	Subc.	5.0	Lived
No. 2....	"	"	5.0	"	"	5.0	Died
No. 3....	"	"	0.0	"	"	5.0	"

Heifer No. 1.—No reaction local or general.
Alive and in good condition, April 1st.
Heifer No. 2.—After 24 hours temp. normal, but local swelling.
After 36 hr. temp. subnormal, swelling developing.
Dead in 40 hours. P. M. Typical Blackleg.

The aggressin injections were made subcutaneously in front of the shoulder. Virus injections were made subcutaneously in the right leg.

TABLE No. 6.

AGGRESSIN IMMUNITY TESTS ON BOVINES.

Heifer	*Immunized*	*Method*	*Aggressin*	*Inoculated*	*Method*	*Virus*	*Result*
No. 4....	2-5-19	Subc.	10.0	3-8-19	Subc.	4.0	Lived
No. 5....	"	"	5.0	"	"	4.0	Died
No. 6....	"	"	0.0	"	"	0.0	"

Heifer No. 4.—No reaction local or general.
Alive and in good condition April 1st.
Heifer No. 5.—After 24 hours, temperature normal, but local swelling.
After 36 hours, temperature normal, but quarter swelling.
Dead in 40 hours. Post mortem—typical blackleg.
Heifer No. 6.—After 18 hours, temperature subnormal, swelling extensive
Dead in 24 hours. Post mortem—typical blackleg.

The results thus obtained from the most recent experiments show clearly the protective value of aggressin against highly active blackleg virus. The virus dose proved to be altogether too high in spite of the attempt to gauge its virulence. There was no question but that the low aggressin animals possessed a positive degree of immunity. Careful post-mortem studies demonstrated that the infection was much less extensive as compared with the control infection, amounting to about one-fourth that of

the unprotected animal. In addition, cultures were obtained from every portion of the body in the normal heifers, but only from the original site of injection in the aggressin heifers. Likewise, the controls all died early and symptoms were more typical. In view of the consistency of the clinical, cultural and pathological pictures, the conviction is firmly accepted that a lethal dosage would have demonstrated perfect immunity in all grades of protected animals.

Encouraging reports are rapidly accumulating in the practical field, giving to the experimental studies a new meaning and stimulating research effort like mental aggressins—making way for success.

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 220, 1919

(Reprint from *Journal of American Veterinary Medical Association,* Vol. LV, New Series, Vol. 8, No. 4, July, 1919, pp. 416-423.)

STUDIES ON ANTHELMINTICS.

I. EXPERIMENTS WITH REPEATED DOSES OF OIL OF CHENOPODIUM.

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The superiority of oil of chenopodium as an ascaricide to other ascaricidal anthelmintics, when suitable doses of the involved drugs are compared, has been experimentally established for the dog by the work of Hall and Foster in the Bureau of Animal Industry, and of Hall in this laboratory, for swine by Hall and Foster (most of the work on swine was done by Foster), and has been clinically established in the case of man by numerous observations of physicians here and in the tropics. In the case of the ascarid of the horse, Hall, Wilson and Wigdor found chenopodium superior to most of the drugs commonly used, and work by Hall, Smead and Wolf, to be published in a paper in this series, shows carbon bisulphid superior to chenopodium.

The therapeutic dose of oil of chenopodium for removing ascarids from dogs has been found by the writer to be 0.1 m. p. k. (mil per kilo). In this dosage, experiments on dogs indicate that it is inferior to chloroform in single therapeutic dose of 0.2-0.3 m. p. k. in removing hookworms. In human medicine, it has been found necessary to give chenopodium in repeated doses, usually at hour intervals, in order to remove hookworms, and even under such conditions, several treatments are not infrequently necessary. To determine the method in which chenopodium could be successfully employed against the hookworm in the dog and to secure further data as to the anthelmintic action of this drug, the following experiments were performed:

*Resigned March 27, 1919.

Chenopodium in Doses Repeated Over a Number of Days.

Dog No. 153, weighing 16 kilos, was given 2 minims of chenopodium in 1 dram of castor oil daily for a total of 12 treatments in 13 days. The dog had distemper and died 2 days after the last treatment. No worms were passed. There were 7 whipworms postmortem. Treatment was therefore 0 percent effective against whipworms.

Dog No. 158, weighing 16 kilos, was given the same treatment daily for a total of 17 treatments in 20 days, and killed the day after the last treatment. The dog passed no worms and had 6 whipworms postmortem. Efficacy against whipworms, 0 percent.

Dog No. 152, weighing 12 kilos, was given the same treatment daily for a total of 18 treatments in 20 days, and killed 3 days after the last treatment. The dog passed 1 hookworm on the twelfth day after beginning treatment. It had 14 *Dipylidium* postmortem. Efficacy against hookworms, 100 per cent; against *Dipylidium,* 0 per cent.

Dog No. 154, weighing 15 kilos, was given the same treatment for a total of 18 treatments in 20 days, and killed 3 days after the last treatment. Through the 8 days after the first treatment, the dog passed a total of 8 hookworms and none thereafter. Postmortem it had 7 hookworms and 41 *Dipylidium.* Efficacy against hookworms, 53 per cent; against *Dipylidium,* 0 percent. It is remarkable that over half of the hookworms should yield to 7 treatments and the remainder resist a total of 18 treatments, but this is in keeping with the difficulties and uncertainties of hookworm treatments as shown in numerous other experiments.

Dog No. 155, weighing 9.5 kilos, was given the same treatment for a total of 18 treatments in 20 days, and was killed 3 days later. In the first 3 days after the first treatment, the dog passed 2 hookworms and 1 was found in the large intestine postmortem. The dog had 2 other hookworms, 19 whipworms and 3 *Dipylidium* postmortem. Efficacy against hookworms, 60 percent; against whipworms and *Dipylidium,* 0 percent. This shows the same peculiarities as regards the resistance of individual hookworms to treatment.

Dog No. 156, weighing 9 kilos, was given the same treatment for a total of 18 treatments in 20 days, and killed 3 days later.

The second day after the first treatment, the dog passed 1 ascarid. No worms were found postmortem. Efficacy against ascarids, 100 percent.

Dog No. 157, weighing 12 kilos, was given the same treatment for a total of 19 treatments in 23 days, and killed 1 day later. The ninth day after the first treatment, the dog passed 1 whipworm. The dog had 16 whipworms postmortem. Efficacy against whipworms, 6 percent.

Dog No. 159, weighing 14 kilos, was given the same treatment for a total of 19 treatments in 23 days, and killed 1 day later. The dog passed no worms and had 1 *Dipylidium* postmortem. Efficacy against *Dipylidium*, 0 percent. The preliminary fecal examination of this dog showed fluke eggs; no flukes were detected in the daily examination of the feces or postmortem. However, these flukes, a species of *Alaria* (*Hemistomum*), are very small, not difficult to detect postmortem, but likely to be destroyed in feces and unrecognizable, and it is likely that the treatment removed them.

Dog No. 162, weighing 12.75 kilos, was given 5 minims of oil of chenopodium in the soft, or soluble elastic, capsule, followed immediately by 2 drams of castor oil, daily, for a total of 12 doses in 13 days, and was killed 5 days later. The second day after the first treatment, the dog passed 1 whipworm. It had 2 *Dipylidium* postmortem. Efficacy against whipworms, 100 percent; against *Dipylidium,* 0 percent.

Dog No. 163, weighing 15 kilos, was given the same treatment for a total of 12 treatments in 13 days, and was killed 5 days later. The third day after the first treatment, the dog passed 1 hookworm, and 1 *Dipylidium* was found in the cecum postmortem. There were 97 other *Dipylidium* postmortem. Efficacy against hookworms, 100 percent; against *Dipylidium,* 1 percent.

Dog No. 164, weighing 15 kilos, was given the same treatment for a total of 12 treatments in 13 days, and was killed 5 days later. The second day after the first treatment, the dog passed 1 hookworm. It had 3 *Dipylidium* postmortem. Efficacy against hookworms, 100 percent; against *Dipylidium,* 0 percent.

Dog No. 165, weighing 14.5 kilos, was given the same treatment for a total of 12 treatments in 13 days, and was killed 2 days later. The fifth day after the first treatment, the dog passed 1

whipworm. It had 7 whipworms and 5 *Dipylidium* postmortem. Efficacy against whipworm, 13 percent; against *Dipylidium,* 0 percent.

Dog No. 166, weighing 12 kilos, was given the same treatment for a total of 12 treatments in 13 days, and was killed 2 days later. In the 5 days after the first treatment, the dog passed 29 ascarids, and in the 3 days after the first treatment, passed 24 *Dipylidium.* It had 1 whipworm postmortem. Efficacy against ascarids and *Dipylidium,* 100 percent; against whipworms, 0 percent.

Dog No. 167, weighing 14.5 kilos, was given 12 treatments in 13 days, and was killed 2 days later. In the 2 days after the first treatment it passed 2 ascarids, and in the 11 days after the first treatment it passed 54 whipworms. It had 12 whipworms and 1 *Dipylidium* postmortem. Efficacy against ascarids, 100 percent; against whipworms, 82 percent; against *Dipylidium,* 0 percent. Owing to an accident while collecting worms postmortem, some whipworms may have been lost, but the efficacy was about 75 percent or more.

Dog No. 168, weighing 11.5 kilos, was given the same treatment for a total of 12 treatments in 13 days, and was killed 2 days later. The day after the first treatment, the dog passed 2 ascarids, and the tenth day after the first treatment it passed 3 whipworms. It had no worms postmortem. Efficacy against ascarids and whipworms, 100 percent.

Dog No. 169, weighing 12 kilos, was given the same treatment for a total of 12 treatments in 13 days, and was killed 2 days later. The day after the first treatment the dog passed 2 ascarids. It had 1 *Dipylidium* postmortem. Efficacy against ascarids, 100 percent; against *Dipylidium,* 0 percent.

Dog No. 283, weighing 11.5 kilos, was given 5 minims of oil of chenopodium in soft (soluble elastic) gelatin capsules, followed by 15 mils of castor oil. Treatment was repeated on the third, fifth and seventh days thereafter. The dog was found dead 3 days after the last treatment. In the 8 days following the first treatment, the dog passed 3 hookworms. It had 11 hookworms postmortem. Efficacy against hookworms, 21 percent.

Dog No. 228, weighing 8 kilos, was given chenopodium at the rate of 0.05 m. p. k., with 30 mils of castor oil. The next day

the dose was repeated and the dog was killed 6 days after the second treatment. On the day following the second treatment the dog passed 5 hookworms, and in the 4 days following the first treatment it passed 31 whipworms. It had 16 hookworms, 175 whipworms, and 2 *Dipylidium* postmortem. Efficacy against hookworms, 24 percent; against whipworms, 15 percent; against *Dipylidium*, 0 percent.

Chenopodium in Doses Repeated During One Day.

(Of the following 7 experiments, the 4 with dogs 293, 294, 299 and 309 have already been published in another paper. They are repeated here for the sake of completion.)

Dog No. 293, weighing 12 kilos, was given one 10-minim soft gelatine capsule of chenopodium every hour for a total of 3 doses, and the last dose was followed an hour later by 15 grams of Epsom salts in simple syrup. During the next 2 days the dog passed 4 hookworms, and was killed the fourth day after treatment. It had no worms postmortem. Efficacy against hookworms, 100 percent.

Dog No. 294, weighing 16 kilos, was given a 10-minim capsule of oil of chenopodium with ⅓ grain of cascarin; this was followed an hour later by a 10-minim capsule of chenopodium and another ⅓ grain of cascarin. In the next 4 days the dog passed 3 hookworms and 1 whipworm. It had 1 hookworm, 21 whipworms, and 6 *Tænia pisiformis* postmortem. Efficacy against hookworms, 75 percent; against whipworms, 5 percent; against tapeworms, 0 percent.

Dog No. 299, weighing 15 kilos, was given one 10-minim soft capsule of chenopodium every hour for a total of 3 doses, and was fed uncooked meat immediately after each dose to see if it afforded protection against the drug in the absence of purgation and also diminished the efficacy. The day after treatment, the dog passed 5 ascarids and 3 hookworms. It was killed the fourth day. It had 5 hookworms. Efficacy against ascarids, 100 percent; against hookworms, 37.5 percent.

Dog No. 314, weighing 6 kilos, was given one 10-minim soft capsule of chenopodium at 8:30 A. M. and immediately fed some uncooked beef heart and bread. At 2:00 P. M. the dog was

given a second 10-minim capsule and offered food, but refused it. At 4:35 it was given a third capsule and food. At 9:00 A. M. the next day the dog was given 30 mils of castor oil. This was an attempt to determine the effects in the administration of a capsule before each meal, advocated by some physicians in human cases of hookworm. The dog passed 2 ascarids the day after treatment, and was killed the fourth day. It had no worms postmortem. Efficacy against ascarids, 100 percent.

Dog No. 309, weighing 14 kilos, was given one 10-minim soft capsule every half-hour for a total of 3 doses, the last dose being followed a half-hour later by 30 mils of castor oil. At an undetermined interval after getting the castor oil, the dog broke out of its cage and ate some food. No worms were passed. The dog was killed the fifth day. It had 2 hookworms and 6 whipworms. Efficacy against hookworms and whipworms, 0 percent.

Dog No. 148, weighing 13 kilos, was given one 10-minim soft capsule of chenopodium every hour for a total of 3 doses, the last dose being followed immediately by 30 mils of castor oil. The dog passed no worms and was killed the fourth day. It had 6 whipworms. Efficacy against whipworms, 0 percent.

Dog No. 151, weighing 13 kilos, was given one 5-minim soft capsule of chenopodium every hour for a total of 3 doses, the last dose being followed immediately by 30 mils of castor oil. The dog passed 1 hookworm the day after treatment and 2 whipworms the third or fourth day after treatment. The animal was killed the fourth day. It had 1 whipworm postmortem. Efficacy against hookworms, 100 percent; against whipworms, 67 percent.

Where very small doses of chenopodium, 2 minims, were given daily for a total of 12 (1 case), 17 (1 case), 18 (4 cases) and 19 (2 cases) treatments, the efficacy against ascarids was, as usual, 100 percent; against hookworms, 100, 60, 53 and 0 percent; against whipworms, 6, 0, 0, 0, and 0 percent; against *Dipylidium*, 0 percent (5 times). These experiments confirm the idea that chenopodium is successful against ascarids almost always, that repeated doses increase its efficacy against hookworms, and that it should not be regarded as a tæniacide. They also bear out the writer's suggestion that santonin is the remedy of choice for whipworms, as even these numerous treatments with small doses of chenopodium fail to remove these worms in most cases.

Where larger doses of chenopodium, 5 minims, are given daily for 12 doses, the drug shows the expected efficacy against ascarids, 100 percent (4 cases); an efficacy against hookworms of 100 percent (2 cases); against whipworms, 100 (2 cases), 75 to 82, 13 and 0 percent; and against tapeworms, 100, 1 and 0 (5 cases) percent. The efficacy against tapeworms is to be expected; the base of 100 percent efficacy against tapeworms is an accident, out of keeping with numerous failures on the part of this drug to remove any tapeworms whatever; the whipworm findings indicate that repeated doses of 5 minims daily are much more effective than 2 minims daily; the findings for hookworm are based in both cases on a single hookworm and so are inconclusive.

A test of this same 5-minim dose every other day for 4 days showed 21 percent efficacy against hookworms.

A test of half the therapeutic dose (0.1 m. p. k.), or 0.05 m. p. k., given on 2 successive days, showed 24 percent efficacy against hookworms, 15 percent against whipworms, and 0 percent against *Dipylidium*.

In the repeated doses given in 1 day, three 10-minim doses at hour intervals, followed by Epsom salts, were 100 percent effective against hookworm, and 67 and 0 percent effective against whipworms; the same dose, given with cascarin, was 75 percent effective against hookworms, 5 percent effective against whipworms, and 0 percent effective against tapeworms; the same dose with meat, but without purgation, was 100 percent effective against ascarids and 37.5 percent effective against hookworms; the same dose, given 3 times during the day with food, was 100 percent effective against ascarids; the same dose, given every half-hour and followed a half-hour later by castor oil, was 0 percent effective against hookworms.

The foregoing suggests that chenopodium in repeated doses of 5 minims daily for 12 doses is rather efficacious against ascarids, whipworms, and, probably, hookworms. But such prolonged treatment is objectionable from the standpoint of the practitioner. The use of three 10-minim doses at hour intervals, followed by a purgative an hour later, gives promise of success in treating dogs as in treating man for hookworms, but, even as in that case, repeated treatments will not infrequently be necessary. This sub-

ject demands more data. Prolonged experience to date only enables us to formulate the following statements with regard to oil of chenopodium:

Oil of chenopodium has no equal as a drug for the removal of ascarids, as it will in the big majority of cases remove 100 percent of the worms present in the dog, and is apparently about as effective, under proper conditions of administration, against ascarids of man and swine. It is apparently as effective as anything against ascarids in the horse, and will probably give satisfactory results when it has been sufficiently studied to ascertain the proper dose and mode of administration.

Chenopodium does not have, in our experience, as much value for removing hookworms in single therapeutic dose as does chloroform, but such experimental evidence as we have, together with the clinical evidence of thousands of human cases treated with chenopodium, indicates that in repeated doses, either at hour intervals or on consecutive days, it should prove reasonably satisfactory against hookworms in dogs.

No drug can be depended on to remove whipworms when given in single dose, as the writer has stated elsewhere. Repeated doses of oil of chenopodium, 5 minims daily for 12 days, for instance, seem to give rather good results and warrant further investigations along this line. But the fact that santonin is not a gastro-intestinal irritant gives it the choice for use against whipworms, so far as we are aware at present. It can be given in doses of a half-grain or a grain daily, with equal amounts of calomel, and seems entirely safe when so given, so far as our experiments show.

Chenopodium will occasionally remove tapeworms, but the numerous failures to remove any in a long series of experiments show positively that it cannot be regarded as a suitable anthelmintic for the removal of tapeworms, so far as dog tapeworms are concerned, and so far as findings in regard to them can be applied to other tapeworms and hosts.

Studies from the Medical Research Laboratories.
Parke, Davis & Co.
Reprint No. 221, 1919.

(Reprinted from *The American Journal of Physiology*, Vol. 49, No. 2, July, 1919.)

The Effect of Feeding Pars Tuberalis and Pars Anterior Proprior of Bovine Pituitary Glands Upon the Early Development of the White Rat.

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INTRODUCTION.

The pituitary body (hypophysis) is usually described as made up of two component parts, the anterior and posterior lobes (1).

Under the general term posterior lobe are included the pars nervosa, the pars intermedia, and the neural stalk. Functionally these structures are very closely related. Extracts of each of them are capable of producing similar characteristic immediate effects when injected into the blood stream. It is believed that the active principles of the posterior lobe are elaborated in the pars intermedia and traverse the pars nervosa and neural stalk to the third ventricle, thus reaching the cerebrospinal fluid (2). The pars intermedia is histologically distinct from the pars nervosa and neural stalk, and traces its embryological origin to an entirely separate anlage. It is, however, very closely joined to the pars nervosa and is an essential part of the posterior lobe.

Injection of extracts prepared from the anterior lobe produce none of the effects characteristic of posterior lobe extracts. Long-continued feeding with the anterior lobe tissue, however, induces certain alterations in development, while the use in a similar way of posterior lobe preparations gives negative results.

Robertson in 1916 published a report in which he states that when fed to growing mice, anterior lobe produced a definite retardation of growth in the period immediately following puberty, this retardation being succeeded by a marked stimulation of growth (5). Goetsch, working with a much smaller number of white rats,

sule a small bit of the pars tuberalis is usually left attached to the pars anterior proprior. This fact opens the possibility that some of the effects of the anterior lobe feeding may be due to the inclusion of small portions of the pars tuberalis in the preparation. In the present study, white rats have been fed upon carefully separated pars tuberalis and pars anterior proprior, in an attempt to answer this question.

TABLE 1

Experiment 1. Gain in body weight (in grams)

SEX		AFTER 6½ WEEKS OF FEEDING			AFTER 12 WEEKS OF FEEDING		
		Group I. Anterior lobe	Group II. Pars tuberalis	Group III. Meat	Group I. Anterior lobe	Group II. Pars tuberalis	Group III. Meat.
Very young females	Number in group	6	2	4			
	Average final weight	72.6	68.0	73.1	Not weighed		
	Average initial weight	16.0	16.7	17.0	because of		
	Average gain	*56.6*	*51.3*	*56.1*	pregnancies		
Older females	Number in group	4	3	3			
	Average final weight	98.2	83 1	97.8	Not weighed		
	Average initial weight	36.4	35.3	38.8	because of		
	Average gain	*61.8*	*47.8*	*59.0*	pregnancies		
Very young males	Number in group		4	2		4	2
	Average final weight		74.7	84.2		90.5	97.0
	Average initial weight		18.2	16.7		18.2	16.7
	Average gain		*56.5*	*67.5*		*72.7*	*80.3*
Older males	Number in group	3	3	3	3	3	3
	Average final weight	113.6	107.1	113.5	130.3	121.3	134.3
	Average initial weight	38 3	38.6	46.0	38.3	38.6	46.0
	Average gain	*75.3*	*68.5*	*67.5*	*92.0*	*82.7*	*88.3*

MATERIAL AND METHODS.

In this experiment albino rats were fed portions of the pituitary gland, and carefully observed for any changes in growth rate and sexual development. The rats used were secured at the age of two to three weeks (shortly after weaning). They were immediately weighed and grouped according to weight and sex. Three separate series of experiments were conducted, one group comprising fifty-three rats, the second, thirty-seven, and the third,

stated that anterior lobe induced a uniform increase of growth-rate, denying the period of retardation (6). Later, Robertson and Delprat reported that anterior lobe feedings definitely increased the prepubertal growth rate of white mice.

The anterior lobe is also known to exert a certain control over the development and proper functioning of the reproductive system. Clinically, pituitary disease is always associated with more or less sexual abnormality, both anatomical and functional (9). Partial extirpation of the anterior lobe in experimental animals is followed by marked hypoplasia of the genitalia (3). Goetsch noted a marked hypertrophy of the genitalia in his pituitary-fed animals (6), while Robertson noted an increase in pugnacity and general virility in his pituitary-fed males (5).

Recently it has been shown that in addition to the structures mentioned above, the hypophysis contains a third epithelial lobe, the pars tuberalis, closely investing the neural stalk (10). The question arises whether this newly described structure is a part of the posterior or of the anterior lobe, or whether it possesses an entirely separate function. From its structural relations it might at first be considered a part of the posterior lobe complex, for it is apparently continuous with the pars intermedia which has been proven to be a part of the posterior lobe mechanism. Atwell (11) has shown, however, that, in the rabbit at least, the pars tuberalis is embryologically quite distinct from the posterior lobe elements, arising as two lateral thickenings of the anterior lobe anlage and only later surrounding the developing neural stalk.

In a recent paper Atwell and Marinus (12) have compared the blood pressure and oxytocic reactions of pars tuberalis extracts with the reactions of pars intermedia, neural stalk and pars nervosa extracts. The reactions elicited by pars tuberalis extracts were so small that they were evidently due to unavoidable contamination of the extracts with active posterior lobe constituents, and not to any inherent activity on the part of pars tuberalis. It was concluded by the authors that the pars tuberalis is not a functional part of the posterior lobe.

Owing to its close anatomical relationship to the anterior lobe it was suspected that the pars tuberalis might be included in the ordinary anterior lobe preparations. Investigations showed that such was the case. As the anterior lobe is rolled from its cap-

ten. The third series contained a single litter born at this laboratory.

During the entire experiment the rats were kept on a standard diet consisting of water and cracked mixed grains in abundance, stale (but not mouldy) bread once a week, and a small quantity of fresh milk daily. The rats were kept in metal cages, without litter except for the grain they scattered about. The cages were

TABLE 2

Experiment 2. Gain in body weight (in grams)

SEX		AFTER 5½ WEEKS OF FEEDING			AFTER 11 WEEKS OF FEEDING		
		Group I. Anterior lobe.	Group II. Pars tuberalis	Group III. Meat.	Group I. Anterior lobe	Group II. Pars tuberalis	Group III. Meat
Very young females	Number in group	3	3	4			
	Average final weight	61.3	61.3	63.2	Not weighed		
	Average initial weight	22.5	27.5	26.1	because of		
	Average gain	*38.8*	*33.8*	*37.1*	pregnancies		
Older females	Number in group	5	4	6			
	Average final weight	94.9	91.2	94.3	Not weighed		
	Average initial weight	44.8	47.1	45.5	because of		
	Average gain	*50.1*	*44.1*	*48.8*	pregnancies		
Very young males	Number in group	7	5	5	7	5	5
	Average final weight	76.4	62.9	73.9	116.4	94.0	109.0
	Average initial weight	29.5	30.1	30.0	29.5	30.1	30.0
	Average gain	*46.9*	*32.8*	*43.9*	*86.9*	*64.1*	*79.0*
Older males	Number in group	4	4	3	4	4	3
	Average final weight	109.4	90.0	101.8	148.5	119.5	138.5
	Average initial weight	46.9	44.0	45.3	46.9	44.0	45.3
	Average gain	62.5	46.0	56.5	101.6	75.5	93.2

cleaned weekly, using a coal-tar disinfectant. Individual rats were distinguished by spotting with stains—methylene blue, acid fuchsin and picric acid proving the most satisfactory. Each rat was weighed twice weekly.

In addition to the standard diet the rats were daily given the experimental foods. A small portion of the fresh gland was presented to the rat in forceps, whereupon it was invariably seized and swallowed. At each feeding it was definitely ascertained that

TABLE 3

Experiment 3. Gain in body weight (in grams). After $6\frac{1}{2}$ weeks of feeding

	Group I. Anterior lobe			Group II. Pars tuberalis			Group III. Meat		
	Initial weight	Final weight	Gain	Initial weight	Final weight	Gain	Initial weight	Final weight	Gain
1. Female	32.0	105.5	73.5	32.0	80.5	48.5	32.5	96.0	63.5
2. Female	35.0	118.5	83.5	34.0	88.5	54.5	33.0	94.0	61.0
3. Male	36.5	129.5	93.0	32.0	100.0	78.0	37.0	112.5	75.5
4. Male				33.5	87.5	74.0			
Total	103.5	353.5	250.0	131.5	350.5	255.0	102.5	302.5	200.0
Average	34.5	117.8	*83.3*	32.9	89.1	*63.7*	34.1	100.8	*66.6*
Relative gain Normal valued at 100			125.0	(cf. table 5)		95.0			100.0

TABLE 4

Experiment 3. Body lengths in centimeters after $6\frac{1}{2}$ weeks of feeding

	Group I. Anterior lobe	Group IV. Pars tuberalis	Group VII. Meat
1. Female	15.2	13.8	13.6
2. Female	16.2	14.8	13.9
3. Male	16.3	14.6	15.2
4. Male		13.6	
Total	47.7	56.7	42.7
Average	15.9	14.2	14.2

TABLE 5

Experiment 3. Weights of genital organs in milligrams

	Group I. Anterior lobe		Group II. Pars tuberalis		Group III. Meat	
	Male	Female	Male	Female	Male	Female
1		160		120		115
2		220		100		140
3	1530		885		910	
4			865			
Average	1530	190	875	110	910	135
Relative variation Normal valued at 100	168	140	96	81	100	100

the portion allotted was completely devoured. The glands used were taken indiscriminately from steer, cow and heifer heads. As soon as possible after the animal was slaughtered the head was split in the sagittal plane and the pituitary gland dissected out. The glands were immediately wrapped in oiled paper and packed in ice. In every case the glands were dissected into their component parts and fed to the rats within six hours after the death of the animal. During this time there was no actual contact between the ice or ice water and the glands. These precautions were taken in order to make certain that the material should lose none of its activity by autolysis, by decomposition or by solution of its constituents in the ice water.

The partes tuberales were dissected out as described by Atwell and Marinus. The material available was then divided into equal portions sufficient to supply all the rats to be fed. Each rat was given a quantity of pars tuberalis which varied from day to day, but which approximated one-fourth of an entire pars tuberalis.

TABLE 6

Experiment 2. Birth of first litter and weight of mother at conception

	GROUP I. ANTERIOR LOBE		GROUP II. PARS TUBERALIS		GROUP III. MEAT	
	Litters	Weight	Litters	Weight	Litters	Weight
7th week of feeding	1	101.0				
8th week	2	94.0				
		90.0				
9th week						
10th week	1	107.5				
11th week	2	91.5				
		121.5			1	107.0
12th week			1	122.0	2	94.0
						89.5
13th week			2	94.5	2	127.0
			(2 preg.)	85.5	(3 preg.)	121.5
Average weight		100.9		100.6		107.8

The anterior lobes were secured by splitting the glands in the midsagittal plane and rolling the entire anterior lobe out of its capsule, leaving the posterior lobe attached to the capsule and dura mater. The portion of the anterior lobe at the point of origin of

the pars tuberalis was snipped off, making certain that no pars tuberalis fluids or tissues were included with the anterior lobe as fed. Each rat was given a portion equal to about one-sixth of the entire lobe (approximately 250 mgm.). As a control certain of the rats were fed beef muscle. A portion of the muscle was freed from all loose fat and was then divided into portions approximately equal in weight to the portions of anterior lobe. All the rats in series 3 were killed with ether after six and one-half weeks of feeding. The body weights and lengths were recorded. The entire female genital tract was dissected out down to the trigone and preserved intact. The sex organs were then fixed in Bouin's fluid and dehydrated in alcohols. When in 80 per cent alcohol each testis (together with the corresponding epididymis) was weighed separately. The female genital tract was weighed as a whole. The tissues were completely dehydrated, cleared with xylol, embedded in paraffin, and cut 5 micra thick. The sections were stained with Heidenhain's iron hematoxylin and eosin.

DISCUSSION.

Puberty occurs in the white rat from sixty to ninety days after birth (13). The rats of experiments 1 and 2 were then barely approaching the normal time of puberty after six and one-half and five and one-half weeks of feeding. This stage corresponds roughly to the end of the second or beginning of the third growth cycle described by Robertson for white mice. Comparison of the weights at this stage shows a small but well defined increase in weight in the anterior-lobe-fed groups over all other groups. This is in agreement with Robertson and Delprat, who conclude that anterior lobe feeding stimulates growth during the second development cycle.

The rats of experiment 3 were born in the same litter and allow more exact comparisons. Here also the anterior-lobe-fed surpassed the other animals at the age of nine weeks (after six and one-half weeks of feeding). The difference is even more marked in this experiment owing to the greater uniformity of the animals.

After twelve weeks of feeding all the females had given birth to at least one litter or were in some stage of pregnancy. The

rats had, therefore, all passed puberty. At this stage, also, the anterior-lobe-fed males were heavier than any of the others, the difference being greater than at the age of puberty. This observation is contrary to Robertson's conclusions that after puberty the administration of anterior lobe to white mice produces a preliminary retardation of growth. The seeming inconsistency may be due to a possible difference in the relative lengths of the growth cycles of white mice and white rats.

The nose-to-anus lengths recorded show that the anterior-lobe-fed rats were considerably larger than either the controls or the pars-tuberalis-fed rats. The increased weights noted in the case of the anterior-lobe-fed rats are, then, due to an increased skeletal growth. This conclusion is in keeping with the increased skeletal growth noted in cases of pathologic hyperpituitarism.

While the anterior-lobe-fed rats were growing faster than the controls, the pars-tuberalis-fed animals were losing ground. In all three experiments the pars-tuberalis-fed rats were markedly smaller than the controls. This was even more constant and more marked than the overgrowth of the anterior-lobe-fed animals. It should be remarked, however, that the pars-tuberalis-fed rats were not strictly controlled. The rats of the other groups received daily a quantity of muscle or glandular tissue weighing approximately 250 mgm., while the tuberalis-fed rats were given only a minute portion of tissue weighing but 5 to 10 mgm.

It is evident that the accelerated growth reported by previous observers as following anterior lobe feeding has not been due to any admixture of pars tuberalis substance, inasmuch as the same effects are produced by anterior lobe material known to be free from other parts of the gland, and inasmuch as no overgrowth is induced by feeding pars tuberalis alone. The apparent retardation of growth must be further studied before it is to be definitely ascribed to pars tuberalis feeding.

In addition to its action on the process of growth, the anterior lobe is known to control, to some extent at least, the development of the reproductive system. The effects of anterior lobe feedings on the sexual organs may be evaluated by *a*, study of the organs themselves, or *b*, date of birth of the first litter. Both methods have been used in this study.

The testes of the anterior-lobe-fed male (experiment 3) were markedly larger than those of the other males, weighing nearly twice as much as the controls. Upon section it was found, as reported by Goetsch, that all of the tissues in the testicle are involved in the hyperplasia but that the increase in size and weight is chiefly attributable to changes in the seminiferous tubules. The female organs were also better developed, although the gross difference was not so marked.

The genital organs of the pars-tuberalis-fed rats could not, however, be distinguished from those of the controls. The slightly greater weight of the control organs is within the limits of individual variation and is explained as concomitant to the larger size of the control animals. Microscopical examination of both testes and ovaries revealed no difference between those of the pars-tuberalis-fed and control rats.

In normal healthy rats the first copulation occurs shortly after the sex glands become histologically mature. Thus the date of birth of the first litter is a good criterion of the relative maturity of two groups of animals. During the ninth week of feeding it became evident that several of the anterior-lobe-fed animals were pregnant, while none of the controls were showing signs of pregnancy. This observation was borne out by the fact that three anterior-lobe-fed females gave birth to the first litters two weeks before any of the control litters were born. The females evidencing this sexual precocity were not larger than the slower controls at the calculated date of impregnation, i. e., the sexual development had been more rapid than the somatic.

The pars-tuberalis-fed and control females dropped their litters during the same period of time, averaging more than two weeks after the anterior-lobe-fed females. In keeping with the microscopical evidence afforded by experiment 3, the births in this experiment show that the pars-tuberalis-feedings do not alter the sexual development or function of the white rat.

SUMMARY AND CONCLUSIONS.

One hundred young rats were separated into three groups. The first group was fed upon pars anterior proprior of the pituitary gland, the second upon pars tuberalis, and the third or control group upon beef muscle. During twelve weeks of feeding the

rats of the first group exhibited increased growth rate accompanied by a more rapid development of the reproductive system, evidenced by gross and microscopical hypertrophy of the organs and by the earlier birth of young. In the second (pars tuberalis fed) group there was no change in the sexual development as compared with that of the control group. The growth rate was slightly slower in the second group, owing perhaps to the smaller amount of meat fed.

This study has not shown that any of the functions ascribed to the anterior lobe as a whole are due to the pars tuberalis.

I wish to express my indebtedness to Dr. Wayne J. Atwell for his valuable assistance in the preparation of this paper.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 222, 1919.

(Reprinted from the *Journal of the American Veterinary Medical Association,* Vol. LV, N. S. Vol. 8, No. 5, August, 1919.)

STUDIES ON ANTHELMINTICS.*

II. THE ANTHELMINTIC AND INSECTICIDAL VALUE OF CARBON BISULPHIDE AGAINST GASTRO-INTESTINAL PARASITES OF THE HORSE.

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By critical experimental methods, *i. e.,* treatment followed by careful examination of the manure and post-mortem examination, it has been found by Hall (1917) that carbon bisulphide is apparently 100 per cent effective against bots; by the same methods, it has been found by Hall, Wilson and Wigdor (1918) that some of the common anthelmintics are not adequately effective against ascarids in the horse, even such drugs as oil of chenopodium, highly ascaricidal for ascarids in other hosts, falling far short of 100 per cent efficacy. Using these same critical methods, we find that carbon bisulphide, in addition to being 100 per cent effective in removing bots, is almost that effective in removing ascarids. This drug has been used heretofore against ascarids in the horse, but in the absence of critical tests, its real efficacy was a problematical quality. We are now able to report that in carbon bisulphide we have a dependable remedy for the refractory ascarid of the horse. This information fills a distinct gap in our knowledge of dependable treatments for parasites of the horse, and in connection with the findings of Hall, Wilson and Wigdor (1918) to the effect that oil of chenopodium, properly used, is approximately 100 per cent effective against strongyles, cylicostomes and pinworms in the horse, it establishes the topic of anthelmintic treatment for the common parasites of the digestive tract of the horse on a sound basis of tested and dependable drugs.

*Read before the Southeastern Michigan Veterinary Medical Association, Detroit, Mich., April 9, 1919.

Our method was the same as that used by Hall, Wilson and Wigdor. The horses were dosed by, and the fasting, feeding and care of manure supervised by, one of us (Smead). The examination of the manure for worms and bots passed and the postmortem examination of the digestive tract were made by Hall, Smead and Wolf, assisted by J. R. Stafford. No effort was made to detect cylicostomes in the manure or to count them postmortem. However, if cylicostomes had been present in the manure to any extent they would probably have been detected, and it is our opinion that practically none were passed.

Food was removed from 8 horses at noon, March 10, 1919, and the animals were given their first, or their only, treatment with carbon bisulphide in hard capsules about 8:30 the next morning. No purgatives were given. In view of the new data relative to the time required for dead bots and worms to pass from a horse under these conditions, we give the protocols rather fully.

Horse No. 1094 was given 6 drams of carbon bisulphide in 1 dose. On the succeeding days, in their order, this horse passed the following: 1 bot, 0 ascarids; 2 bots, 0 ascarids; 58 bots, 1 ascarid; total 61 bots, 1 ascarid. The horse was killed the third day after treatment and had 105 dead bots and 5 dead ascarids in the large intestine on their way out. The drug removed 166 bots and 6 ascarids, leaving none in the stomach or small intestine. This horse had 85 pinworms, hundreds of *Strongylus* spp. and thousands of *Cylicostomum* spp. The treatment was therefore 100 per cent effective against bots and ascarids and 0 per cent effective against pinworms, *Strongylus* spp. and *Cylicostomum* spp. (We assume from the number of cylicostomes left, together with the failure to remove *Strongylus* and pinworms, that the treatment was an entire failure against cylicostomes, even though the manure was not closely examined to see if any of these were passed.) The stomach and small intestine were normal.

Horse No. 1093 was also given 6 drams of carbon bisulphide in 1 dose. On the succeeding days this horse passed bots as follows: 0 bots; 8 bots; 70 bots; 77 bots; 36 bots; 48 bots; 33 bots; 5 bots; 2 bots; total, 279 bots. This horse passed 2 ascarids on the third day after treatment and 2 or 3 at a later day; owing to a misunderstanding, exact records were not kept for these worms. The horse was killed the ninth day after treatment and had 2 bots

in the double colon and no ascarids anywhere. This horse had 1 pinworm, hundreds of *Strongylus* spp. and some *Cylicostomum* spp. The treatment was therefore 100 per cent effective against bots and ascarids and 0 per cent effective against pinworms, *Strongylus* spp. and *Cylicostomum* spp. The stomach showed a healing inflamed area in the cardiac portion.

Horse No. 1092 was given 4 drams of carbon bisulphide at 1 dose and this dose was repeated 2 hours later. On the succeeding days, in their order, this horse passed the following: 0 bots, 0 ascarids; 0 bots, 1 ascarid; 0 bots, 4 ascarids; total, 0 bots, 5 ascarids. The horse was killed the third day after treatment and had no bots anywhere; it had 2 live ascarids in the small intestine and 38 dead ones in the large intestine. There were 9 *Strongylus* spp., some *Cylicostomum* spp. and no pinworms. The treatment was therefore over 95 per cent effective against ascarids and 0 per cent effective against *Strongylus* and *Cylicostomum* spp.; no data regarding bots and pinworms, as these parasites were not present. Cardiac stomach was inflamed and showed adherent mucous exudate.

Horse No. 823 was also given 2 4-dram doses of carbon bisulphide at a 2-hour interval. On the succeeding days this horse passed the following: 1 bot, 0 ascarids; 0 bots, 2 ascarids; 1 bot, 10 ascarids; 1 bot, 4 ascarids; 0 bots, 6 ascarids; 0 bots, 3 ascarids; 0 bots, 0 ascarids; 0 bots, 1 ascarid; total 3 bots, 26 ascarids. Subsequent to the eighth day after treatment, no bots or ascarids were passed. The horse was killed on the seventeenth day after treatment and had no bots or ascarids anywhere. There were hundreds of *Strongylus,* thousands of *Cylicostomum* and no pinworms. The treatment was, therefore, 100 per cent effective against bots and ascarids, and 0 per cent effective against *Strongylus* and *Cylicostomum.* In passing, it may be noted that the small number of bots present in this horse is correlated with the fact that this animal had not been on pasture the previous summer, but had been kept in the stable or allowed in a bare lot for exercise. The inflammation of the gastric mucosa, following treatment, had almost entirely subsided.

Horse No. 1091 was also given 2 4-dram doses of carbon bisulphide at a 2-hour interval. On the succeeding days this horse passed bots as follows: 0, 7, 13, 6, 4, 2, 2, 0, 1, 0, 0, 0, 0, 0;

total 35 bots. This horse also passed 3 ascarids, but the exact date was not recorded. The horse was killed the fourteenth day after treatment and had no bots or ascarids post-mortem; it had hundreds of *Strongylus* spp. and numerous *Cylicostomum* spp., but no pinworms. The treatment was therefore 100 per cent effective against bots and ascarids, but 0 per cent effective against *Strongylus* and, apparently, *Cylicostomum*. There are no conclusions regarding pinworms, as these were not present. There had been some inflammation in the cardiac stomach, but this had almost entirely subsided at the time of necropsy.

Horse No. 897 was also given 2 4-dram doses of carbon bisulphide at a 2-hour interval. On the succeeding days, this horse passed no bots. At some date it passed 1 or 2 ascarids, but, owing to a misunderstanding, no records of the number of worms or the date were kept. The horse was killed on the fourteenth day after treatment and was found to have no bots. There was 1 live ascarid in the small intestine. The horse had 4 pinworms, numerous *Strongylus* and some *Cylicostomum*. The treatment was not entirely successful against ascarids in this case, removing 1 or more and leaving 1. This is probably due to the worm being in the lower ileum and the drug being largely absorbed before reaching the site of the worm. The treatment was 0 per cent effective against pinworms, *Strongylus* and, apparently, *Cylicostomum*. There are no conclusions in regard to bots, as there were none present. This freedom from bots is correlated with the fact that this animal had been kept off pasture the preceding summer. The stomach of this animal showed evidence of an inflammation, in the cardiac portion, that had almost entirely subsided.

Horse No. 1100 was given 3 doses of 3 drams each of carbon bisulphide at 1-hour intervals. On the succeeding days this horse passed the following: 0 bots, 0 ascarids; 31 bots, 4 ascarids; 58 bots, 2 ascarids; 29 bots, 0 ascarids; 14 bots, 0 ascarids; 13 bots, 0 ascarids; total 145 bots, 6 ascarids. The horse was killed the sixth day after treatment and had 15 dead bots in the large intestine and no ascarids anywhere. There were 4 pinworms, hundreds of *Strongylus* spp. and some *Cylicostomum* spp. The treatment was therefore 100 per cent effective against bots and ascarids and 0 per cent effective against pinworms, *Strongylus* spp. and *Cylicostomum* spp. A considerable portion of the cardiac stomach was highly inflamed.

Horse No. 1106 was given the same treatment, 3 doses of 3 drams each at 1-hour intervals. On the succeeding days this horse passed the following: 0 bots, 0 ascarids; 17 bots, 1 ascarid; 12 bots, 1 ascarid; 7 bots, 0 ascarids; 6 bots, 0 ascarids; 1 bot, 0 ascarids; 0 bots, 0 ascarids; 0 bots, 0 ascarids; 0 bots, 0 ascarids; 1 bot, 0 ascarids; total 44 bots, 2 ascarids. After the tenth day no parasites were passed. The animal was killed on the seventeenth day. One dead bot was found in the double colon and no ascarids anywhere. There were numerous *Strongylus*, innumerable *Cylicostomum* and no pinworms. Treatment was, therefore, 100 per cent effective against bots and ascarids, and 0 per cent effective against *Strongylus* and, apparently, *Cylicostomum*. The inflammation of the gastric mucosa had almost entirely subsided.

A consideration of the foregoing shows the following:

Carbon bisulphide has a dependable efficacy of approximately 100 per cent against bots and ascarids, the two common and important parasites occurring in the anterior portion of the digestive tract, *i. e.*, the stomach and small intestine, of the horse. In our animals, the bots were mostly *Gastrophilus nasalis*, with a few *G. hemorrhoidalis;* the ascarids were the common *Ascaris equorum* (*A. megalocephala*). In our experiments, it was uniformly 100 per cent effective against bots, removing all of 690 bots from the 6 infested animals, and usually 95 to 100 per cent effective against ascarids, removing (approximately) 91 of 94 worms from the 8 infested animals, or almost 97 per cent.

Carbon bisulphide gives as good results in 1 6-dram dose as in 2 4-dram doses or 3 3-dram doses, and it is likely that the smaller sum total of drug in the one dose is to be preferred to the greater total in several doses, especially as the gastric lesions seem less pronounced with the 1 dose. It is quite possible that further experiment will show that a single dose even smaller than 6 drams will be adequate. Dove (1918) found that young bots could be killed by carbon bisulphide in 45 minutes, while last-stage larvæ required almost 3½ hours, *G. intestinalis* being more resistant than other species. The question as to whether carbon bisulphide in one dose remains in the stomach long enough to remove the bots, and perhaps remains as much as 3½ hours, would seem to be answered in the affirmative by the success of our 1-dose treatment.

Carbon bisulphide given without purgation will remove the bots, but they will usually not be found in the manure for the first 24 hours after treatment, and the maximum number are apt to be in the manure of the third day, and may be in that of the fourth day after treatment. Dead bots may be passed for 10 days and others may still be present in the large intestine 17 days after treatment. Where purgation is employed, bots may come away in the first 24 hours, according to Dove (1918), usually the following day, however, and may come away for 5 days, according to the findings of Hall (1917) and Dove (1918).

Ascarids usually come away on the second and third day, but may come away as late as the eighth day.

Carbon bisulphide is of no value against worms in the posterior portion of the digestive tract, the cecum, colon and rectum, *i. e.,* against pinworms, *Strongylus* and *Cylicostomum.* This is perhaps due to the rapid absorption of the drug in the stomach and small intestine, and this may account for the occasional escape of an ascarid located in the lower portion of the small intestine. The simultaneous administration of linseed oil might serve to carry the carbon bisulphide in solution down the intestine more rapidly, increasing its efficacy against ascarids. Whether it would cause the removal of any worms from the large intestine is doubtful. Hall (1919) has reported two experiments where a 20-mil dose of carbon bisulphide, or two such doses at 2-hour intervals, followed in 1½ to 2½ hours by 800 mils of linseed oil, entirely failed to remove any strongyles from horses.

In this connection, it may be said that the carbon bisulphide is soluble in oils, but is practically insoluble in water; Dove (1918) is in error in stating: "The carbon bisulphide, being soluble in water, evidently reaches all portions of the stomach, either as a gas or in solution." A common laboratory test for iodine is that employing a discrete undissolved bubble of CS_2 at the bottom of an aqueous solution.

Obviously, adequate anthelmintic treatment for removal of all the common species of worms and bots from the horse would require consecutive treatments with carbon bisulphide and oil of chenopodium, the two anthelmintics now known to be dependable for the purpose.

The lesion due to carbon bisulphide given in hard capsules

consists in inflammation in the cardiac portion of the stomach, usually over an area the size of a man's hand or larger. This inflammation, when present, subsides almost entirely in the course of two weeks. The fact that horse No. 1094 had a normal stomach on the third day after treatment with 1 6-dram dose, suggests that this single-dose treatment occasions less local damage than repeated doses; certainly the amount of toxic drug absorbed is less.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 223, 1919.

(Reprinted from the *Journal of the American Veterinary Medical Association,* Vol. LV, N. S. Vol. 8, No. 6, September, 1919.)

STUDIES ON ANTHELMINTICS.

III. CHLOROFORM AS AN ANTHELMINTIC.

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That chloroform is valuable as an anthelmintic for removing hookworms—what we may call an uncinaricide—has been claimed by Allesandrini on the basis of clinical experience with human patients, and has been experimentally shown in tests against hookworms of the dog by Schultz and by Hall and Foster. Chloroform is a constituent, and the active uncinaricidal ingredient, of Hermann's mixture, which contains chloroform and castor oil combined with oleoresin of male fern or oil of eucalyptus, and is also present in a number of proprietary and trade preparations.

In experiments on dogs, Hall and Foster found that 0.1 m. p. k. (mil per kilo) was too small a dose for this drug, and experiments in this laboratory indicate that at least 0.2 or 0.3 m. p. k. should be used. This naturally raises the question as to whether chloroform is a safe drug to use and what constitutes a safe therapeutic dose and a lethal dose.

It has long been known that the administration of chloroform to produce anesthesia was sometimes followed by a delayed poisoning in which pathological alterations of the liver were a prominent condition, sometimes accompanied by degenerations of the kidneys and heart. Bevan and Favill referred the toxic effects of delayed chloroform poisoning to toxins which could not be eliminated from the liver cells. They noted the presence in the blood and urine of acetone, diacetic acid and betaoxybutyric acid. Opie and Alford found that fats increased in susceptibility to chloroform poisoning, while

*Resigned March 27, 1919.

carbohydrates afforded a measure of protection against it, which accords with the clinical experience of English surgeons. Offergeld thought that death in delayed chloroform poisoning is due to nephrolysis, as a result of the action of the chloroform on the kidney cells. Wells thought that the liver changes are due to the effect on the liver of poisons that destroy the synthetic functions of the liver cells without destroying their autolytic ferments, with a resultant autolysis of the liver cells, indicated by the presence of free amino acids, purins, proteoses, peptones and polypeptids in the liver. Whipple and Hurwitz have found that fibrinogen may be almost eliminated from the circulating blood in chloroform poisoning, subsequently reappearing after the repair of the liver, which takes place usually within 10 days. Whipple and Sperry find that chloroform anesthesia for one or two hours invariably causes some central liver necrosis and note that chloroform anesthesia for 35 minutes in man may cause almost complete liver necrosis with a fatal termination; the essential change is extensive necrosis and fatty degeneration of the liver; there may be numerous ecchymoses and hemorrhages into the peritoneum and upper intestinal tract; the pancreas may show fat necrosis and ecchymoses; in pregnancy there may be placental necrosis with separation and hemorrhages; the liver necrosis is microscopically visible only after 6 to 10 hours; they find that dogs die in 1 to 4 days, with symptoms of intoxication and vomiting, sometimes vomiting blood. Schoenhof has summarized 29 cases of delayed chloroform poisoning in man, of which 17 recovered and 12 were fatal. He cites some experiments on dogs as follows:

Dog 1, weighing 5.5 kilos, was given 7 mils of chloroform (a little over 1.27 m. p. k.); after 5 minutes dog was restless, salivated and staggering; recovered in a half hour. Next day, this dog was given 20 mils of chloroform (a little over 3.63 m. p. k.); symptoms more pronounced, dog vomiting and falling; dullness, sleepiness and vomiting of blood lasted 2 days; dog recovered. This animal's total dose was practically 5 mils per kilo.

Dog 3, weighing 4.5 kilos, was given 50 mils of a mixture of absolute alcohol (28 parts), chloroform (40 parts), and

camphor (20 parts); died in 15 minutes. The chloroform dosage here is 5 mils per kilo, without considering the other drugs used, and the other drugs must have contributed largely to the toxic effects.

Dog 4, a medium-sized dog (he regards a dog weighing 5.5 kilos as a medium-sized dog in a previous experiment; Hall and Wigdor found a 10-kilo dog to be the average size) was given 35 mils of chloroform (about 6 m. p. k. apparently); dog vomited and was dull; the next day the dog was given a mixture of 5 mils of alcohol and 20 mils of chloroform (apparently about 3.6 m. p. k.) and died almost immediately. Apparently this dog had almost 10 mils per kilo, in addition to the alcohol.

Graham believes that the necrosis following chloroform is due to its breakdown in the body with the formation of hydrochloric acid, a belief supported by the increase of salts in the urine and by the inability of ether and chloral hydrate to parallel the changes caused by chloroform, dichlormethane and tetrachlormethane; alkalis inhibit the production of these lesions. Farquhar has used sodium bicarbonate by mouth, by rectum, subcutaneously and intravenously, in a very serious case of delayed chloroform poisoning in man, with recovery, which bears out Graham's findings. Jensen finds adrenalin valuable in cases of chloroform poisoning. Whipple and Speed find that in normal animals the liver eliminates from 45 to 65 per cent of the phenoltetrachlorphthalein injected intravenously, but where the liver is damaged, as by chloroform, phthalein appears in the urine; in one of their experiments, a 21-pound dog (a little less than 10 kilos) was given 15 mils of chloroform, followed 4½ hours later by 10 mils additional, a total of over 2.5 mils per kilo; the dog was still alive 4 days afterward (no further data given). Simonds finds that feeding sugar in cases of phosphorus or chloroform poisoning furnishes easily oxidized material to the liver, in which the glycogen is depleted, inhibiting autolysis.

It appears from the experiments noted in the foregoing, that dogs may survive the oral administration of 5 mils of chloroform per kilo of body weight, when given in 2 doses on successive days. Evidently doses of 0.3 mils per kilo should

be readily tolerated as a therapeutic dose of chloroform by dogs in reasonably good condition.

The following experiments were performed in this laboratory:

Dog No. 19, weighing 22 kilos, was given chloroform at the rate of 0.1 m. p. k. in 30 mils of castor oil. The dog passed no worms; was killed the fourth day; had 1 ascarid, 3 hookworms, and 12 tapeworms. Efficacy against ascarids, hookworms and tapeworms, 0 per cent. Digestive tract in bad condition.

Dog No. 12, weighing 6 kilos, was given chloroform at the rate of 0.2 m. p. k. in 30 mils of castor oil. The dog passed 7 hookworms; was killed the fourth day after treatment; had 1 hookworm and 6 tapeworms postmortem. Efficacy against hookworms, 87.5 per cent; against tapeworms, 0 per cent. Digestive tract in good condition.

Dog No. 89, weighing 9 kilos, was given chloroform at the rate of 0.2 m. p. k. in 30 mils of castor oil. The dog passed 6 hookworms; was killed the twentieth day; had 75 hookworms and 3 ascarids. Efficacy against hookworms, 7 per cent; against ascarids, 0 per cent. Digestive tract in good condition.

Dog No. 115, weighing 10.5 kilos, was given chloroform at the rate of 0.3 m. p. k. in 30 mils of castor oil. The dog passed 2 hookworms; was killed the fifth day; had 2 hookworms postmortem. Efficacy against hookworms, 50 per cent. Digestive tract in fair condition.

Dog No. 118, weighing 12.5 kilos, was given chloroform at the rate of 0.3 m. p. k. in 30 mils of castor oil. The dog passed 1 hookworm; was killed the seventh day; had 2 hookworms, 3 whipworms and 43 tapeworms postmortem. Efficacy against hookworms, 33 per cent; against whipworms and tapeworms, 0 per cent. Digestive tract in fair condition.

Dog No. 58, weighing 3 kilos, was given chloroform at the rate of 0.4 m. p. k. in 30 mils of castor oil. The dog passed no worms; died the second day after treatment; had no worms postmortem. Digestive tract in bad condition. This dog was a weak pup, which accounts for its failure to tolerate this dose.

Dog No. 83, weighing 9.5 kilos, was given chloroform at the rate of 0.4 m. p. k. in 30 mils of castor oil, and 5 hours

the pars tuberalis was snipped off, making certain that no pars tuberalis fluids or tissues were included with the anterior lobe as fed. Each rat was given a portion equal to about one-sixth of the entire lobe (approximately 250 mgm.). As a control certain of the rats were fed beef muscle. A portion of the muscle was freed from all loose fat and was then divided into portions approximately equal in weight to the portions of anterior lobe. All the rats in series 3 were killed with ether after six and one-half weeks of feeding. The body weights and lengths were recorded. The entire female genital tract was dissected out down to the trigone and preserved intact. The sex organs were then fixed in Bouin's fluid and dehydrated in alcohols. When in 80 per cent alcohol each testis (together with the corresponding epididymis) was weighed separately. The female genital tract was weighed as a whole. The tissues were completely dehydrated, cleared with xylol, embedded in paraffin, and cut 5 micra thick. The sections were stained with Heidenhain's iron hematoxylin and eosin.

DISCUSSION.

Puberty occurs in the white rat from sixty to ninety days after birth (13). The rats of experiments 1 and 2 were then barely approaching the normal time of puberty after six and one-half and five and one-half weeks of feeding. This stage corresponds roughly to the end of the second or beginning of the third growth cycle described by Robertson for white mice. Comparison of the weights at this stage shows a small but well defined increase in weight in the anterior-lobe-fed groups over all other groups. This is in agreement with Robertson and Delprat, who conclude that anterior lobe feeding stimulates growth during the second development cycle.

The rats of experiment 3 were born in the same litter and allow more exact comparisons. Here also the anterior-lobe-fed surpassed the other animals at the age of nine weeks (after six and one-half weeks of feeding). The difference is even more marked in this experiment owing to the greater uniformity of the animals.

After twelve weeks of feeding all the females had given birth to at least one litter or were in some stage of pregnancy. The

rats had, therefore, all passed puberty. At this stage, also, the anterior-lobe-fed males were heavier than any of the others, the difference being greater than at the age of puberty. This observation is contrary to Robertson's conclusions that after puberty the administration of anterior lobe to white mice produces a preliminary retardation of growth. The seeming inconsistency may be due to a possible difference in the relative lengths of the growth cycles of white mice and white rats.

The nose-to-anus lengths recorded show that the anterior-lobe-fed rats were considerably larger than either the controls or the pars-tuberalis-fed rats. The increased weights noted in the case of the anterior-lobe-fed rats are, then, due to an increased skeletal growth. This conclusion is in keeping with the increased skeletal growth noted in cases of pathologic hyperpituitarism.

While the anterior-lobe-fed rats were growing faster than the controls, the pars-tuberalis-fed animals were losing ground. In all three experiments the pars-tuberalis-fed rats were markedly smaller than the controls. This was even more constant and more marked than the overgrowth of the anterior-lobe-fed animals. It should be remarked, however, that the pars-tuberalis-fed rats were not strictly controlled. The rats of the other groups received daily a quantity of muscle or glandular tissue weighing approximately 250 mgm., while the tuberalis-fed rats were given only a minute portion of tissue weighing but 5 to 10 mgm.

It is evident that the accelerated growth reported by previous observers as following anterior lobe feeding has not been due to any admixture of pars tuberalis substance, inasmuch as the same effects are produced by anterior lobe material known to be free from other parts of the gland, and inasmuch as no overgrowth is induced by feeding pars tuberalis alone. The apparent retardation of growth must be further studied before it is to be definitely ascribed to pars tuberalis feeding.

In addition to its action on the process of growth, the anterior lobe is known to control, to some extent at least, the development of the reproductive system. The effects of anterior lobe feedings on the sexual organs may be evaluated by *a*, study of the organs themselves, or *b*, date of birth of the first litter. Both methods have been used in this study.

The testes of the anterior-lobe-fed male (experiment 3) were markedly larger than those of the other males, weighing nearly twice as much as the controls. Upon section it was found, as reported by Goetsch, that all of the tissues in the testicle are involved in the hyperplasia but that the increase in size and weight is chiefly attributable to changes in the seminiferous tubules. The female organs were also better developed, although the gross difference was not so marked.

The genital organs of the pars-tuberalis-fed rats could not, however, be distinguished from those of the controls. The slightly greater weight of the control organs is within the limits of individual variation and is explained as concomitant to the larger size of the control animals. Microscopical examination of both testes and ovaries revealed no difference between those of the pars-tuberalis-fed and control rats.

In normal healthy rats the first copulation occurs shortly after the sex glands become histologically mature. Thus the date of birth of the first litter is a good criterion of the relative maturity of two groups of animals. During the ninth week of feeding it became evident that several of the anterior-lobe-fed animals were pregnant, while none of the controls were showing signs of pregnancy. This observation was borne out by the fact that three anterior-lobe-fed females gave birth to the first litters two weeks before any of the control litters were born. The females evidencing this sexual precocity were not larger than the slower controls at the calculated date of impregnation, i. e., the sexual development had been more rapid than the somatic.

The pars-tuberalis-fed and control females dropped their litters during the same period of time, averaging more than two weeks after the anterior-lobe-fed females. In keeping with the microscopical evidence afforded by experiment 3, the births in this experiment show that the pars-tuberalis-feedings do not alter the sexual development or function of the white rat.

SUMMARY AND CONCLUSIONS.

One hundred young rats were separated into three groups. The first group was fed upon pars anterior proprior of the pituitary gland, the second upon pars tuberalis, and the third or control group upon beef muscle. During twelve weeks of feeding the

rats of the first group exhibited increased growth rate accompanied by a more rapid development of the reproductive system, evidenced by gross and microscopical hypertrophy of the organs and by the earlier birth of young. In the second (pars tuberalis fed) group there was no change in the sexual development as compared with that of the control group. The growth rate was slightly slower in the second group, owing perhaps to the smaller amount of meat fed.

This study has not shown that any of the functions ascribed to the anterior lobe as a whole are due to the pars tuberalis.

I wish to express my indebtedness to Dr. Wayne J. Atwell for his valuable assistance in the preparation of this paper.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 222, 1919.

(Reprinted from the *Journal of the American Veterinary Medical Association*, Vol. LV, N. S. Vol. 8, No. 5, August, 1919.)

STUDIES ON ANTHELMINTICS.*

II. THE ANTHELMINTIC AND INSECTICIDAL VALUE OF CARBON BISULPHIDE AGAINST GASTRO-INTESTINAL PARASITES OF THE HORSE.

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By critical experimental methods, *i. e.*, treatment followed by careful examination of the manure and post-mortem examination, it has been found by Hall (1917) that carbon bisulphide is apparently 100 per cent effective against bots; by the same methods, it has been found by Hall, Wilson and Wigdor (1918) that some of the common anthelmintics are not adequately effective against ascarids in the horse, even such drugs as oil of chenopodium, highly ascaricidal for ascarids in other hosts, falling far short of 100 per cent efficacy. Using these same critical methods, we find that carbon bisulphide, in addition to being 100 per cent effective in removing bots, is almost that effective in removing ascarids. This drug has been used heretofore against ascarids in the horse, but in the absence of critical tests, its real efficacy was a problematical quality. We are now able to report that in carbon bisulphide we have a dependable remedy for the refractory ascarid of the horse. This information fills a distinct gap in our knowledge of dependable treatments for parasites of the horse, and in connection with the findings of Hall, Wilson and Wigdor (1918) to the effect that oil of chenopodium, properly used, is approximately 100 per cent effective against strongyles, cylicostomes and pinworms in the horse, it establishes the topic of anthelmintic treatment for the common parasites of the digestive tract of the horse on a sound basis of tested and dependable drugs.

*Read before the Southeastern Michigan Veterinary Medical Association, Detroit, Mich., April 9, 1919.

Our method was the same as that used by Hall, Wilson and Wigdor. The horses were dosed by, and the fasting, feeding and care of manure supervised by, one of us (Smead). The examination of the manure for worms and bots passed and the postmortem examination of the digestive tract were made by Hall, Smead and Wolf, assisted by J. R. Stafford. No effort was made to detect cylicostomes in the manure or to count them postmortem. However, if cylicostomes had been present in the manure to any extent they would probably have been detected, and it is our opinion that practically none were passed.

Food was removed from 8 horses at noon, March 10, 1919, and the animals were given their first, or their only, treatment with carbon bisulphide in hard capsules about 8:30 the next morning. No purgatives were given. In view of the new data relative to the time required for dead bots and worms to pass from a horse under these conditions, we give the protocols rather fully.

Horse No. 1094 was given 6 drams of carbon bisulphide in 1 dose. On the succeeding days, in their order, this horse passed the following: 1 bot, 0 ascarids; 2 bots, 0 ascarids; 58 bots, 1 ascarid; total 61 bots, 1 ascarid. The horse was killed the third day after treatment and had 105 dead bots and 5 dead ascarids in the large intestine on their way out. The drug removed 166 bots and 6 ascarids, leaving none in the stomach or small intestine. This horse had 85 pinworms, hundreds of *Strongylus* spp. and thousands of *Cylicostomum* spp. The treatment was therefore 100 per cent effective against bots and ascarids and 0 per cent effective against pinworms, *Strongylus* spp. and *Cylicostomum* spp. (We assume from the number of cylicostomes left, together with the failure to remove *Strongylus* and pinworms, that the treatment was an entire failure against cylicostomes, even though the manure was not closely examined to see if any of these were passed.) The stomach and small intestine were normal.

Horse No. 1093 was also given 6 drams of carbon bisulphide in 1 dose. On the succeeding days this horse passed bots as follows: 0 bots; 8 bots; 70 bots; 77 bots; 36 bots; 48 bots; 33 bots; 5 bots; 2 bots; total, 279 bots. This horse passed 2 ascarids on the third day after treatment and 2 or 3 at a later day; owing to a misunderstanding, exact records were not kept for these worms. The horse was killed the ninth day after treatment and had 2 bots

in the double colon and no ascarids anywhere. This horse had 1 pinworm, hundreds of *Strongylus* spp. and some *Cylicostomum* spp. The treatment was therefore 100 per cent effective against bots and ascarids and 0 per cent effective against pinworms, *Strongylus* spp. and *Cylicostomum* spp. The stomach showed a healing inflamed area in the cardiac portion.

Horse No. 1092 was given 4 drams of carbon bisulphide at 1 dose and this dose was repeated 2 hours later. On the succeeding days, in their order, this horse passed the following: 0 bots, 0 ascarids; 0 bots, 1 ascarid; 0 bots, 4 ascarids; total, 0 bots, 5 ascarids. The horse was killed the third day after treatment and had no bots anywhere; it had 2 live ascarids in the small intestine and 38 dead ones in the large intestine. There were 9 *Strongylus* spp., some *Cylicostomum* spp. and no pinworms. The treatment was therefore over 95 per cent effective against ascarids and 0 per cent effective against *Strongylus* and *Cylicostomum* spp.; no data regarding bots and pinworms, as these parasites were not present. Cardiac stomach was inflamed and showed adherent mucous exudate.

Horse No. 823 was also given 2 4-dram doses of carbon bisulphide at a 2-hour interval. On the succeeding days this horse passed the following: 1 bot, 0 ascarids; 0 bots, 2 ascarids; 1 bot, 10 ascarids; 1 bot, 4 ascarids; 0 bots, 6 ascarids; 0 bots, 3 ascarids; 0 bots, 0 ascarids; 0 bots, 1 ascarid; total 3 bots, 26 ascarids. Subsequent to the eighth day after treatment, no bots or ascarids were passed. The horse was killed on the seventeenth day after treatment and had no bots or ascarids anywhere. There were hundreds of *Strongylus,* thousands of *Cylicostomum* and no pinworms. The treatment was, therefore, 100 per cent effective against bots and ascarids, and 0 per cent effective against *Strongylus* and *Cylicostomum*. In passing, it may be noted that the small number of bots present in this horse is correlated with the fact that this animal had not been on pasture the previous summer, but had been kept in the stable or allowed in a bare lot for exercise. The inflammation of the gastric mucosa, following treatment, had almost entirely subsided.

Horse No. 1091 was also given 2 4-dram doses of carbon bisulphide at a 2-hour interval. On the succeeding days this horse passed bots as follows: 0, 7, 13, 6, 4, 2, 2, 0, 1, 0, 0, 0, 0, 0;

total 35 bots. This horse also passed 3 ascarids, but the exact date was not recorded. The horse was killed the fourteenth day after treatment and had no bots or ascarids post-mortem; it had hundreds of *Strongylus* spp. and numerous *Cylicostomum* spp., but no pinworms. The treatment was therefore 100 per cent effective against bots and ascarids, but 0 per cent effective against *Strongylus* and, apparently, *Cylicostomum*. There are no conclusions regarding pinworms, as these were not present. There had been some inflammation in the cardiac stomach, but this had almost entirely subsided at the time of necropsy.

Horse No. 897 was also given 2 4-dram doses of carbon bisulphide at a 2-hour interval. On the succeeding days, this horse passed no bots. At some date it passed 1 or 2 ascarids, but, owing to a misunderstanding, no records of the number of worms or the date were kept. The horse was killed on the fourteenth day after treatment and was found to have no bots. There was 1 live ascarid in the small intestine. The horse had 4 pinworms, numerous *Strongylus* and some *Cylicostomum*. The treatment was not entirely successful against ascarids in this case, removing 1 or more and leaving 1. This is probably due to the worm being in the lower ileum and the drug being largely absorbed before reaching the site of the worm. The treatment was 0 per cent effective against pinworms, *Strongylus* and, apparently, *Cylicostomum*. There are no conclusions in regard to bots, as there were none present. This freedom from bots is correlated with the fact that this animal had been kept off pasture the preceding summer. The stomach of this animal showed evidence of an inflammation, in the cardiac portion, that had almost entirely subsided.

Horse No. 1100 was given 3 doses of 3 drams each of carbon bisulphide at 1-hour intervals. On the succeeding days this horse passed the following: 0 bots, 0 ascarids; 31 bots, 4 ascarids; 58 bots, 2 ascarids; 29 bots, 0 ascarids; 14 bots, 0 ascarids; 13 bots, 0 ascarids; total 145 bots, 6 ascarids. The horse was killed the sixth day after treatment and had 15 dead bots in the large intestine and no ascarids anywhere. There were 4 pinworms, hundreds of *Strongylus* spp. and some *Cylicostomum* spp. The treatment was therefore 100 per cent effective against bots and ascarids and 0 per cent effective against pinworms, *Strongylus* spp. and *Cylicostomum* spp. A considerable portion of the cardiac stomach was highly inflamed.

Horse No. 1106 was given the same treatment, 3 doses of 3 drams each at 1-hour intervals. On the succeeding days this horse passed the following: 0 bots, 0 ascarids; 17 bots, 1 ascarid; 12 bots, 1 ascarid; 7 bots, 0 ascarids; 6 bots, 0 ascarids; 1 bot, 0 ascarids; 0 bots, 0 ascarids; 0 bots, 0 ascarids; 0 bots, 0 ascarids; 1 bot, 0 ascarids; total 44 bots, 2 ascarids. After the tenth day no parasites were passed. The animal was killed on the seventeenth day. One dead bot was found in the double colon and no ascarids anywhere. There were numerous *Strongylus*, innumerable *Cylicostomum* and no pinworms. Treatment was, therefore, 100 per cent effective against bots and ascarids, and 0 per cent effective against *Strongylus* and, apparently, *Cylicostomum*. The inflammation of the gastric mucosa had almost entirely subsided.

A consideration of the foregoing shows the following:

Carbon bisulphide has a dependable efficacy of approximately 100 per cent against bots and ascarids, the two common and important parasites occurring in the anterior portion of the digestive tract, *i. e.*, the stomach and small intestine, of the horse. In our animals, the bots were mostly *Gastrophilus nasalis*, with a few *G. hemorrhoidalis*; the ascarids were the common *Ascaris equorum* (*A. megalocephala*). In our experiments, it was uniformly 100 per cent effective against bots, removing all of 690 bots from the 6 infested animals, and usually 95 to 100 per cent effective against ascarids, removing (approximately) 91 of 94 worms from the 8 infested animals, or almost 97 per cent.

Carbon bisulphide gives as good results in 1 6-dram dose as in 2 4-dram doses or 3 3-dram doses, and it is likely that the smaller sum total of drug in the one dose is to be preferred to the greater total in several doses, especially as the gastric lesions seem less pronounced with the 1 dose. It is quite possible that further experiment will show that a single dose even smaller than 6 drams will be adequate. Dove (1918) found that young bots could be killed by carbon bisulphide in 45 minutes, while last-stage larvæ required almost 3½ hours, *G. intestinalis* being more resistant than other species. The question as to whether carbon bisulphide in one dose remains in the stomach long enough to remove the bots, and perhaps remains as much as 3½ hours, would seem to be answered in the affirmative by the success of our 1-dose treatment.

Carbon bisulphide given without purgation will remove the bots, but they will usually not be found in the manure for the first 24 hours after treatment, and the maximum number are apt to be in the manure of the third day, and may be in that of the fourth day after treatment. Dead bots may be passed for 10 days and others may still be present in the large intestine 17 days after treatment. Where purgation is employed, bots may come away in the first 24 hours, according to Dove (1918), usually the following day, however, and may come away for 5 days, according to the findings of Hall (1917) and Dove (1918).

Ascarids usually come away on the second and third day, but may come away as late as the eighth day.

Carbon bisulphide is of no value against worms in the posterior portion of the digestive tract, the cecum, colon and rectum, *i. e.*, against pinworms, *Strongylus* and *Cylicostomum*. This is perhaps due to the rapid absorption of the drug in the stomach and small intestine, and this may account for the occasional escape of an ascarid located in the lower portion of the small intestine. The simultaneous administration of linseed oil might serve to carry the carbon bisulphide in solution down the intestine more rapidly, increasing its efficacy against ascarids. Whether it would cause the removal of any worms from the large intestine is doubtful. Hall (1919) has reported two experiments where a 20-mil dose of carbon bisulphide, or two such doses at 2-hour intervals, followed in 1½ to 2½ hours by 800 mils of linseed oil, entirely failed to remove any strongyles from horses.

In this connection, it may be said that the carbon bisulphide is soluble in oils, but is practically insoluble in water; Dove (1918) is in error in stating: "The carbon bisulphide, being soluble in water, evidently reaches all portions of the stomach, either as a gas or in solution." A common laboratory test for iodine is that employing a discrete undissolved bubble of CS_2 at the bottom of an aqueous solution.

Obviously, adequate anthelmintic treatment for removal of all the common species of worms and bots from the horse would require consecutive treatments with carbon bisulphide and oil of chenopodium, the two anthelmintics now known to be dependable for the purpose.

The lesion due to carbon bisulphide given in hard capsules

consists in inflammation in the cardiac portion of the stomach, usually over an area the size of a man's hand or larger. This inflammation, when present, subsides almost entirely in the course of two weeks. The fact that horse No. 1094 had a normal stomach on the third day after treatment with 1 6-dram dose, suggests that this single-dose treatment occasions less local damage than repeated doses; certainly the amount of toxic drug absorbed is less.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 223, 1919.

(Reprinted from the *Journal of the American Veterinary Medical Association*, Vol. LV, N. S. Vol. 8, No. 6, September, 1919.)

STUDIES ON ANTHELMINTICS.

III. CHLOROFORM AS AN ANTHELMINTIC.

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That chloroform is valuable as an anthelmintic for removing hookworms—what we may call an uncinaricide—has been claimed by Allesandrini on the basis of clinical experience with human patients, and has been experimentally shown in tests against hookworms of the dog by Schultz and by Hall and Foster. Chloroform is a constituent, and the active uncinaricidal ingredient, of Hermann's mixture, which contains chloroform and castor oil combined with oleoresin of male fern or oil of eucalyptus, and is also present in a number of proprietary and trade preparations.

In experiments on dogs, Hall and Foster found that 0.1 m. p. k. (mil per kilo) was too small a dose for this drug, and experiments in this laboratory indicate that at least 0.2 or 0.3 m. p. k. should be used. This naturally raises the question as to whether chloroform is a safe drug to use and what constitutes a safe therapeutic dose and a lethal dose.

It has long been known that the administration of chloroform to produce anesthesia was sometimes followed by a delayed poisoning in which pathological alterations of the liver were a prominent condition, sometimes accompanied by degenerations of the kidneys and heart. Bevan and Favill referred the toxic effects of delayed chloroform poisoning to toxins which could not be eliminated from the liver cells. They noted the presence in the blood and urine of acetone, diacetic acid and betaoxybutyric acid. Opie and Alford found that fats increased in susceptibility to chloroform poisoning, while

*Resigned March 27, 1919.

carbohydrates afforded a measure of protection against it, which accords with the clinical experience of English surgeons. Offergeld thought that death in delayed chloroform poisoning is due to nephrolysis, as a result of the action of the chloroform on the kidney cells. Wells thought that the liver changes are due to the effect on the liver of poisons that destroy the synthetic functions of the liver cells without destroying their autolytic ferments, with a resultant autolysis of the liver cells, indicated by the presence of free amino acids, purins, proteoses, peptones and polypeptids in the liver. Whipple and Hurwitz have found that fibrinogen may be almost eliminated from the circulating blood in chloroform poisoning, subsequently reappearing after the repair of the liver, which takes place usually within 10 days. Whipple and Sperry find that chloroform anesthesia for one or two hours invariably causes some central liver necrosis and note that chloroform anesthesia for 35 minutes in man may cause almost complete liver necrosis with a fatal termination; the essential change is extensive necrosis and fatty degeneration of the liver; there may be numerous ecchymoses and hemorrhages into the peritoneum and upper intestinal tract; the pancreas may show fat necrosis and ecchymoses; in pregnancy there may be placental necrosis with separation and hemorrhages; the liver necrosis is microscopically visible only after 6 to 10 hours; they find that dogs die in 1 to 4 days, with symptoms of intoxication and vomiting, sometimes vomiting blood. Schoenhof has summarized 29 cases of delayed chloroform poisoning in man, of which 17 recovered and 12 were fatal. He cites some experiments on dogs as follows:

Dog 1, weighing 5.5 kilos, was given 7 mils of chloroform (a little over 1.27 m. p. k.); after 5 minutes dog was restless, salivated and staggering; recovered in a half hour. Next day, this dog was given 20 mils of chloroform (a little over 3.63 m. p. k.); symptoms more pronounced, dog vomiting and falling; dullness, sleepiness and vomiting of blood lasted 2 days; dog recovered. This animal's total dose was practically 5 mils per kilo.

Dog 3, weighing 4.5 kilos, was given 50 mils of a mixture of absolute alcohol (28 parts), chloroform (40 parts), and

camphor (20 parts); died in 15 minutes. The chloroform dosage here is 5 mils per kilo, without considering the other drugs used, and the other drugs must have contributed largely to the toxic effects.

Dog 4, a medium-sized dog (he regards a dog weighing 5.5 kilos as a medium-sized dog in a previous experiment; Hall and Wigdor found a 10-kilo dog to be the average size) was given 35 mils of chloroform (about 6 m. p. k. apparently); dog vomited and was dull; the next day the dog was given a mixture of 5 mils of alcohol and 20 mils of chloroform (apparently about 3.6 m. p. k.) and died almost immediately. Apparently this dog had almost 10 mils per kilo, in addition to the alcohol.

Graham believes that the necrosis following chloroform is due to its breakdown in the body with the formation of hydrochloric acid, a belief supported by the increase of salts in the urine and by the inability of ether and chloral hydrate to parallel the changes caused by chloroform, dichlormethane and tetrachlormethane; alkalis inhibit the production of these lesions. Farquhar has used sodium bicarbonate by mouth, by rectum, subcutaneously and intravenously, in a very serious case of delayed chloroform poisoning in man, with recovery, which bears out Graham's findings. Jensen finds adrenalin valuable in cases of chloroform poisoning. Whipple and Speed find that in normal animals the liver eliminates from 45 to 65 per cent of the phenoltetrachlorphthalein injected intravenously, but where the liver is damaged, as by chloroform, phthalein appears in the urine; in one of their experiments, a 21-pound dog (a little less than 10 kilos) was given 15 mils of chloroform, followed 4½ hours later by 10 mils additional, a total of over 2.5 mils per kilo; the dog was still alive 4 days afterward (no further data given). Simonds finds that feeding sugar in cases of phosphorus or chloroform poisoning furnishes easily oxidized material to the liver, in which the glycogen is depleted, inhibiting autolysis.

It appears from the experiments noted in the foregoing, that dogs may survive the oral administration of 5 mils of chloroform per kilo of body weight, when given in 2 doses on successive days. Evidently doses of 0.3 mils per kilo should

be readily tolerated as a therapeutic dose of chloroform by dogs in reasonably good condition.

The following experiments were performed in this laboratory:

Dog No. 19, weighing 22 kilos, was given chloroform at the rate of 0.1 m. p. k. in 30 mils of castor oil. The dog passed no worms; was killed the fourth day; had 1 ascarid, 3 hookworms, and 12 tapeworms. Efficacy against ascarids, hookworms and tapeworms, 0 per cent. Digestive tract in bad condition.

Dog No. 12, weighing 6 kilos, was given chloroform at the rate of 0.2 m. p. k. in 30 mils of castor oil. The dog passed 7 hookworms; was killed the fourth day after treatment; had 1 hookworm and 6 tapeworms postmortem. Efficacy against hookworms, 87.5 per cent; against tapeworms, 0 per cent. Digestive tract in good condition.

Dog No. 89, weighing 9 kilos, was given chloroform at the rate of 0.2 m. p. k. in 30 mils of castor oil. The dog passed 6 hookworms; was killed the twentieth day; had 75 hookworms and 3 ascarids. Efficacy against hookworms, 7 per cent; against ascarids, 0 per cent. Digestive tract in good condition.

Dog No. 115, weighing 10.5 kilos, was given chloroform at the rate of 0.3 m. p. k. in 30 mils of castor oil. The dog passed 2 hookworms; was killed the fifth day; had 2 hookworms postmortem. Efficacy against hookworms, 50 per cent. Digestive tract in fair condition.

Dog No. 118, weighing 12.5 kilos, was given chloroform at the rate of 0.3 m. p. k. in 30 mils of castor oil. The dog passed 1 hookworm; was killed the seventh day; had 2 hookworms, 3 whipworms and 43 tapeworms postmortem. Efficacy against hookworms, 33 per cent; against whipworms and tapeworms, 0 per cent. Digestive tract in fair condition.

Dog No. 58, weighing 3 kilos, was given chloroform at the rate of 0.4 m. p. k. in 30 mils of castor oil. The dog passed no worms; died the second day after treatment; had no worms postmortem. Digestive tract in bad condition. This dog was a weak pup, which accounts for its failure to tolerate this dose.

Dog No. 83, weighing 9.5 kilos, was given chloroform at the rate of 0.4 m. p. k. in 30 mils of castor oil, and 5 hours

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 226, 1919.

(Reprinted from the *American Journal of Pharmacy*, Vol. 91, No. 9, September, 1919.)

THE USE OF ANIMALS IN THE DEVELOPMENT AND STANDARDIZATION OF MEDICINAL PRODUCTS.

By Herbert C. Hamilton.

(From the Medical Research Laboratories, Parke, Davis & Co., Detroit, Mich.)

"The Meyers bill, just introduced in Congress, making it a crime to vivisect the dog, carries into the open and vociferous forum of public debate a question that has disturbed the friendly relations of doctors and dog-lovers for decades.

"There are all shades and degrees of opinion on the subject, all coming at last to the prime consideration of whether it is right under any circumstances to endanger or take the life of one living being for the sake of another.

"So many of the sound principles of modern surgery have been established by experimentation on dogs, cats, monkeys and other animals, to the very great benefit of human beings, it will be rather hard for the anti-vivisectionists to hold their ground, particularly if they are still eaters of chops from the innocent lamb and steaks from the cunning calf."*

In searching for a logical reason for the animus against vivisection one must conclude that it is based on one or the other of two ideas; first, that the use of animals in colleges and research laboratories is unnecessary and avoidable; second, that such use of animals is needlessly cruel and inhuman.

Regarding the first of these it should be sufficient to point out that while many of the valuable medicaments were undoubtedly discovered and their values determined by observation of their effects on humans, research men are not now so favorably placed; it is rare indeed when one can find a person willing to be the first on whom a substance which may or may not be a therapeutic agent, shall be tried. The risk is too great, for the toxicity may be greater than its medicinal value.

Regarding the second of these, while in some cases a prelim-

*Editorial, *The Detroit Journal.*

mary anesthesia is not possible, in a large percentage of the cases where animals are employed for ascertaining the effect of a drug, the observation can be made much more carefully, and more accurate deductions drawn if the factor of pain be excluded entirely.

It should be noted further that the use of animals in research is always with the hope of learning facts which will be helpful in relieving pain or saving lives, either human or animal.

This cannot be said of the great destruction of animal life by hunters and trappers who wound or kill for pleasure and only incidentally for profit; a profit which is almost always personal.

It cannot be said of the slaughter of animals for food, since animal food is not essential to life.

It cannot be said of the slaughter of animals for bounty, since in many cases the losses caused by certain animals on whose head a price is set is less than those losses due to an abnormal increase in the numbers of other predatory animals which had formerly been their prey.

Another point not to be overlooked is that in some cases, notably that of using dogs, the demand for research uses rarely equals the supply of undesirables which would have been killed with no useful return, while in other cases, such as guinea-pigs, rats and mice, the normal supply must be constantly augmented by intensive breeding, in order to meet the demand for research and testing purposes. The use of dogs is therefore no economic loss, and the death no more painful in general than the form of death decreed by the municipality.

General statements, such as those made above, are of little value in combating the untruths and half truths of the anti-vivisectionist. In no other way than by a general investigation can the falsity and absurdity of many of their statements be established.

It has seemed best to meet the situation by a history of the development of certain products, and the methods of testing, together with an emphasis on the value to the world, on the one hand, of an intimate knowledge of the properties of medicinal substances, and on the other hand the benefit resulting from accurate knowledge of the life processes in health and in disease. One must start with certain premises, such, for example, as these:

That some medicinal substances are valuable for relieving pain or prolonging life; that other valuable agents may be discovered

or developed, substances possibly more valuable than those with which we are familiar; that most human lives are more valuable than the average animal's life. It follows, therefore, that almost any number of animals might be used in developing a remedy for tuberculosis, for example, which, in the U. S. alone, has a toll of 150,000 lives yearly.

There is still another side to the picture—the saving of animal life. Hog cholera in one year, in one State of the Union, caused a loss of nearly 3,000,000 hogs, while one hog, when hyperimmunized with the virus, will supply serum sufficient to immunize 100 hogs.

The disease of rinderpest in South Africa has been practically exterminated by following the information gained from animal experimentation. It is a disease from which hundreds of thousands of cattle died annually, but the antivivisectionist sees only the rabbits that were inoculated to study the disease and the remedial measures, while the suffering and death among millions of infected cattle are overlooked.

What is the answer? What of experiments to eliminate Texas fever in cattle, white scours in calves, chicken cholera, dog distemper? When a disease causes an economic loss and waste of valuable life, experiments are undertaken to eliminate or control the disease, whether it is human or animal life. Vivisection in its broader application therefore saves the lives of thousands of animals to one that it takes.

Antitoxin for diphtheria required the use of a large number of animals before it was perfected, but its use has reduced the mortality from over 80 per cent. to under 20 per cent. of those attacked. While in some cases improved hygienic measures may be equally responsible with the remedial agent for a lowered death rate, there is, practically, no other treatment for diphtheria than the administration of antitoxin.

In preparing antitoxin by developing it in the horse's blood, a certain number of horses are valueless for this purpose because the immune bodies will not develop or will acquire only a nominal potency. How is this to be determined? On your child or mine? Or is it more humane to standardize the serum on guinea-pigs and by this means eliminate antitoxin of low potency which is indistinguishable from a potent sample by any known test except that on the living animal or human being?

Recently in the daily press, we read of the men who, in the interests of the army as a whole, served as a means of demonstrating whether or not trench fever is transmissible by "cooties." While this disease may not be regarded in the same category as some, during the war it involved discomfort and possible death to many a soldier. This method of study was necessary because it is impossible by animal experiment to demonstrate the correctness of the theory.

The world proclaims as a hero the physician who risks his life to verify certain facts, such as the transmissibility of yellow fever and malaria by the bite of the mosquito. If it were possible to substitute the life of a dog or a horse for that of the man with equal benefit to the world, would it be difficult to choose? The average person would say that the physician was the more valuable to the community and the world.

The natives of Africa were able to make their weapons more effective by dipping the arrow points into a poison prepared from certain seeds, finely ground, and partially extracted. Careful study of this poison on animals revealed the fact that it has a peculiar effect on the heart, causing, in sublethal doses, a slowing and strengthening of the heart-beat.

In certain cardiac diseases, where the pulse is weak and rapid, it is logical to use strophanthus, the arrow poison, to counteract the abnormal condition. No statistics could be collected to show the value of this remedial agent, discovered by the hunter and developed by the pharmacologist, the vivisector, but its constant use indicates its importance. Today, although its place is secure as one of the most valuable of the heart tonics, strophanthus still exacts a certain toll of lives. Why? Because the physician prefers that some almost worthless frogs should die than that he should use an overdose of this powerful drug.

One of the most valuable phases of animal experimentation is the elimination of harmful and of valueless drugs, giving the physician greater assurance of obtaining the desired results from administration of remedies. From this also has resulted the substitution of the pure principles for the crude drugs and nauseous extracts previously used.

It is a common occurrence to find on the market strophanthus seed as low in activity as one-fourth that of the adopted standard,

and, on the other hand, two or three times as active as this standard. An under dose, as, for example, a dose from a sub-standard preparation, might be harmless, but if a life were hanging by a thread and required a dose of an active heart stimulant, either a highly potent or a worthless sample would be equally fatal. In the one case, the worthless sample would not stimulate the heart muscles to the necessary activity. In the other, an extract of an exceptionally potent lot of drug would poison the heart by overstimulation and just as surely cause death.

Ergot is another illustration of this. It has been used for centuries in aiding childbirth and arresting hemorrhage, but has suffered often from the fact that it is uncertain in its action, some extracts being apparently devoid of any action on the uterus. Within my memory, a German chemist put on the market the supposedly active principle called clavin. This substance might be tested in two ways, either in cases of labor on human subjects, or by a few careful tests on anesthetized animals. Two or three tests by the latter method were sufficient to show that clavin was quite inert.

Further chemical, combined with pharmacological, investigations established the fact that ergot owes its therapeutic value to three active principles each of which has its well-marked effect and each of which is equally essential to the complete action of the drug. Without animal experiments carried out in connection with the chemical investigations the composition of ergot and its rational use would still be uncertain. Even with our present knowledge of its composition we are still unable to standardize extracts of ergot except by a physiological test. The usual test applied, however, does not involve death, anesthesia or even suffering except the prick of the hypodermic needle.

The uncertain effects of extracts of cannabis indica, before physiological experiments established the fact that this is due to variable quality, almost discredited the drug as a therapeutic agent. The question may logically be asked why chemical tests should not be applied to standardize medicinal substances. In reply to this, it may be sufficient to say that pharmacologic standardization (vivisection is one of its broader aspects) is applied only where the active agent is of such a character that chemical tests cannot be applied. It is a more expensive and a less accurate means of standardization and in no case will be retained after an accurate chemical test has been developed.

Pellagra, scurvy, beriberi, and some other less well-marked diseases of undernourishment, have to a large extent been of necessity studied on human subjects. In that way observers have learned that certain foods are essential, but that certain forms of food seem deficient in nourishment. In one historic instance a group of Dr. Goldberger's assistants—16 in all, of whom 13 were physicians—voluntarily submitted themselves to experiments in order to demonstrate whether the symptoms of pellagra could be reproduced by any method of infection. The materials used were blood, nasopharyngeal secretions, epidermal scales from skin lesions, urine and feces. These were administered by injections, by application to the mucosa of nose and pharynx, and by mouth. The evidence while negative led to the conclusion that the disease is one due to faulty diet. Assuming that a corrected diet will eliminate one of the worst scourges of the South, who will deny that these physiological experiments were worth while? On the same principle and even with greater humaneness, the use of animals is to be commended wherever such use is possible.

The development of vitamins is another triumph in which physiological studies have suggested the introduction of a valuable therapeutic agent. By experiments on pigeons, rats and guinea-pigs, it is possible to demonstrate a life process which could be only inferred from observations on humans, to prove that certain foods are deficient although supposedly containing all the essential constituents. In scurvy, the lacking factor is found in certain fresh foods or in lemon or lime juice; in pellagra, it is apparently in part the substance in the shell or germ of the corn which is removed in milling; in beriberi, it is a loss of the substance removed in polishing rice, or in certain conditions occurring when foods are prepared.

In humans, the disease is slowly developed and responds only slowly to treatment, since it is usually complicated by accompanying pathological processes not directly connected with the disease. In pigeons, on the other hand, the condition of polyneuritis can be developed in a remarkably short time by a diet consisting largely of polished rice, while the recovery from this condition takes place in only a few hours when an extract of the polishings of the rice or, better still, an extract of yeast, is administered. The chemical characteristics of these various therapeutic agents are as indefinite

as the exact status each has in nutrition, but the deficiency of each of them in the diet can now be recognized and remedial agents suggested, based on the experiments conducted by a number of prominent physiologists, as Funk, Eijkman, Veddar, Hopkins, Goldberger and many others. The possible value of this work in recognizing and overcoming disease due to nutritional deficiencies is immeasurable.

Koller, an ophthalmologist of Vienna, in 1884 tested the anesthetizing properties of cocaine on guinea-pigs, rabbits and dogs. After noting that in these animals the eye could be touched or scratched without pain after a 2 per cent. solution had been dropped into it, he tried the same solution on man, and thus was introduced into medicine a new method of relieving pain and permitting of operations, previously unendurable.

Cocaine is an exceedingly valuable local anesthetic, but it is highly toxic and has besides a habit-forming action which greatly restricts its use. As a result of a careful chemical study of its composition and structure, combined with pharmacological experiments on animals, it has become possible entirely to eliminate this drug as a local anesthetic, substituting a substance almost equally efficient but at the same time much less toxic and with no habit-forming effects. This in itself would justify the practice of vivisection.

In many surgical operations, profuse bleeding is almost unavoidable, even with the utmost precautions. Further, there are many individuals whose blood is very slow in coagulating and with whom an operation is regarded as almost surely fatal. By the aid of animal experimentation and from the blood and tissues of other animals, substances have been produced which when introduced into the circulating blood shorten its coagulation time so greatly that the danger from excessive hemorrhage has been largely eliminated even in those cases known as hemophiliacs.

Without vivisection such results could scarcely have been obtained. The dog, the horse, the cow and the goat contribute to this valuable therapeutic agent. The part taken by the dog is that of test animal to determine whether the active agents are present, since no known chemical test will show whether they are present in an active form. The dog is, therefore, no less essential in the cycle of operations than the other animals employed. There is no ap-

parent reason why the dog, especially the stray, which spreads disease, contracts and transmits rabies, kills sheep and is rarely useful, should be protected, while an open season exists for deer, quail and trout, and there is no closed season for many animals more deserving of protection.

The animals mostly used in pharmacologic experiments—vivisection, if you please to call it that—are the frog, mouse, rat, guinea-pig, rabbit, dog, cat and monkey. Of these, the frog owes its value to the fact that being a cold-blooded animal, its isolated tissues survive a considerable time and can therefore be used, for example, for the study of muscular contraction and the function of the nerves. The guinea-pig, rat, mouse and rabbit are chiefly of value for inoculation experiments; while the cat, dog and monkey are useful particularly for experiments on the brain, central nervous system and circulatory system.

The dog is especially valuable in nutrition and digestion experiments because of its diet, which is as varied as that of man. For many purposes no other animal can be used on account of the size of the organs and the convenience of handling. For blood-pressure experiments in studying the heart tonics of the digitalis series, standardizing extracts of the pituitary and suprarenal glands, testing the efficiency of hemostatics and blood-coagulating agents, standardizing hypnotics, such as cannabis indica, chloral, chloretone and similar substances, no other animal is so well adapted and from no other animal can the results be transferred directly, almost without alteration, to man.

Where pain would accompany the experiment, and when this point is not the subject of the experiment, a preliminary anesthesia with chloretone is usually applied. This general anesthetic can be given internally by mouth and is often so used. Complete anesthesia, recognized by the absence of reflex when the cornea is touched, results in a half-hour. Anesthesia remains complete, when the proper dose is used, until death, the animal being killed at the end of the experiment by a lethal dose of digitalis.

There are certain historical cases where animal experimentation did not precede human use, as for example, Sir Robert Christianson, who almost died from eating Calabar bean from which physostigmine comes; Koeppe, who tried the effects of digitoxin on himself, with like result. Chloroform and prussic acid were

also investigated with equally unpleasant results. In some cases deaths have occurred, particularly in cases of infectious diseases.

Animal experiments were used to explain caisson disease and suggest means for its prevention and remedy, animals being subjected to air pressure and the pressure applied and released under varying conditions. Such experiments have also made possible the surgical operations which have done so much to relieve pain and prolong life, the human being having the benefit of experience and skill gained by operations on animals.

While physiological experimentation and the standardization of drugs require the use of all the animals mentioned, the dog is more widely used and is almost beyond replacement. Every one, almost without exception, regards the dog as a highly intelligent animal, a fit companion for man. There are few dogs used in research laboratories that would have the appeal of Mark Twain's "Tale of a Dog" or that would be welcomed by any but the small boy. The class of dogs used in experiments, picked up by the dog-catcher and not redeemed, is almost without exception friendless. Even the antivivisectionist would at most feel only pity for it, and with proper recognition of the use to which it is put would probably realize that in a remarkably few cases is any cruelty involved in using it as a test animal.

Most people will agree with Darwin in saying that "cruelty to the lower animals is worthy of detestation and contempt." But what is cruelty? The transportation and preparation of animals for food, the method of slaughter, hunting, fishing, trapping, are often cruel to an extreme. But they are not condemned. More actual cruelty is probably practiced in this way in a season than occurs in all the research laboratories in the world in a year. Can vivisection be condemned and sport exonerated?

The advancement of knowledge, the mitigation of misery and the prevention of disease are surely infinitely higher and nobler motives for infliction of pain, when pain is actually inflicted, than merely healthful exercise and transient enjoyment.

Is it logical, therefore, to attempt legislation to limit and restrict a form of research so valuable to mankind?

Studies from the Medical Research Laboratories.
Parke, Davis & Co.
Reprint No. 227, 1919.

(Reprinted from the *Medical Record*, September 27, 1919.)

THE PRESENT ASPECTS OF ENDOCRINOLOGY.*

By A. W. Lescohier, M.D.

(From the Medical Research Laboratories, Parke, Davis & Co., Detroit, Mich.)

The therapeutics of endocrine glands constitutes a phase of medical practice which has distinctly suffered from a lack of correlated laboratory and clinical study. The scientific side of endocrinology, as reflected by the results of physiological and pathological investigation, has to a large extent been divorced from the application of glandular extracts on a therapeutic basis. Experimental study has established the relationship of various members of the endocrine group to both normal and pathological physiology, so that it is now possible to interpret definitely important symptom complexes in terms of internal secretory disorders. Unfortunately, however, the therapeutic aspects of the subject have not been guided by the same scrutinizing consideration. Empiricism, rather than scientific study, has been the outstanding factor in the use of ductless glands in treatment, and this sort of therapy, from being one of the most delicate phases of medical treatment, has often assumed the earmarks of a patent-medicine "cure-all."

This situation has resulted in the development of two abnormal viewpoints on the part of medical men toward the therapeutic application of glandular products. On the one hand we have the not insignificant proportion of medical practitioners ready to feed on the last rumor or fad, whose soaring imagination has been quick to embrace every wild hypothesis advanced in connection with glandular treatment, and to convert immediately dubious conjectures into established facts. In contradistinction to this class we have the other extreme of bald scepticism, a natural result of the reaction on the part of the conservative element to the faddist

*Read at the twentieth annual meeting of the American Therapeutic Society at Atlantic City, June 6 and 7, 1919.

enthusiasm. The last-mentioned viewpoint frankly questions the value of glandular therapeutics except in a few clear-cut applications such as the thyroid in cretinism, and the employment of suprarenal and pituitary derivatives on a drug basis.

While deprecating the extravagant ideas of the unbalanced enthusiast, it must be conceded, on the other hand, that there is an unwarranted tincture of pessimism in certain of the criticisms which have been leveled at glandular therapy. There are positive as well as negative facts to be remembered, and the real usefulness of our knowledge of the internal secreting glands is largely to be measured by the degree in which it can be utilized in treatment.

The crying need of endocrinology at present is carefully controlled therapeutic investigation. While there is a great deal still to be learned about the physiology and pathology of these diseases there is no dearth of investigation in this direction; indeed, as has previously been suggested, the scientific study of the ductless glands has been dominated by the laboratory investigator, and such carefully controlled study as has been devoted to the clinical side of this question has been almost entirely focused on the striking and unusual types of secretory aberration, which, after all, are probably the least important in looking at the therapeutics of these disorders from a broad viewpoint. The relation of endocrine glands to such conditions as Graves' disease and acromegaly is interesting, but offers very little in the way of practical therapeutics. On the other hand, it seems probable that in the less distinct, the commonly unrecognized functional deficiencies, lies an important field of glandular therapy. The physician who is trained to recognize the less marked endocrine aberrations is the exception and not the rule. By way of illustration it may be pointed out that the diagnosis of cretinism and myxedema is not difficult, and the results from thyroid treatment in their control are definite and generally accepted. The physician does not, however, meet with cases of this type with any great frequency, while on the other hand the milder types of hypothyrodism are by no means uncommon.

The physician who has been taught to recognize the thyroid deficiencies will find this factor associated in many of the clinical conditions which he is called upon to treat. It may be found as a complicating factor in various nervous conditions, in menstrual

disturbances, in anemia, chlorosis, and as a secondary factor in various chronic diseases. Thyroid insufficiency in pregnancy is quite a common occurrence. Cases of obesity exhibiting physical and mental sluggishness and an inability to maintain mental concentration, especially if associated with a dry, cold skin, may be attributed to hypothyroidism. Fatigue from slight exertion in individuals exhibiting good muscular development may be an evidence of thyroid insufficiency. These less distinct types of hypofunction constitute a much larger field for glandular therapeutics than the well-known thyroid syndromes, although the application of such treatment is not, of course, so vitally important.

Certain disturbances of the female generative system attributable to endocrine secretory aberrations constitute another group of general interest. With the exception of thyroid therapeutics there is, perhaps, no other phase of endocrinology in which the relationships are more definitely expressed and the opportunities for treatment more clearly indicated. The influence of ovarian activity on the menstrual function has long been recognized, and finds expression in many ways. More recently we have come to know that other glands, especially the thyroid, the pituitary, and the mammary, may affect the development or influence the functional activities of the sex organs.

It is not the purpose of this paper to review the physiology of or to discuss the interrelationships between these various glandular components, but merely to emphasize a few facts important from a therapeutic standpoint. Obviously, the rational field for glandular treatment includes those cases which are correlated with secretory deficiency.

The best-known application of ovarian treatment is the use of this gland in its entirety, or more often a preparation of the corpora lutea, in controlling the disturbances incident to either natural or artificial menopause. The results of glandular treatment in these conditions are not as consistent as we might naturally expect in view of the certainty that many of the symptoms, at least, are attributable to the disappearance of ovarian secretion. The failure in some of these cases is undoubtedly to be attributed to the complicating glandular factors; in other words, the disturbances of the menopause, whether occurring physiologically or as the result of removal or destruction of the ovaries, are to be interpreted as

a general disturbance of the endocrine balance, in which the dominating factor is subject to variation.

The results of ovarian treatment are undoubtedly more consistent in certain disorders of the menstrual function. In so far as menstruation depends upon ovarian activity, we might expect that absence or scantiness of the menses would be associated with secretory deficiency, and conversely, that excessive or difficult menstruation would be the expression of hyperfunction. This is to a considerable extent substantiated by clinical experience, but other factors may be involved, especially in menorrhagia or dysmenorrhea, conditions in which the secretory element is perhaps less important than certain other factors.

The relation of ovarian activity is more frequent and positive in the disorders of the deficiency type; that is, amenorrhea or scanty and infrequent menses. This group of cases includes women who are physically underdeveloped, and whose menses, from puberty, have been irregular or scant; those in whom the menstrual function has been normal but subsequently becomes irregular or deficient, and who perhaps for long periods of time do not menstruate at all; and cases of amenorrhea or scanty menstruation associated with hysterical temperament. In these last cases the prognosis of glandular therapy depends upon whether the hysterical condition is referable to glandular hyposecretion, or to an inherent instability of the nervous system. It is not to be expected that organotherapy will be successful in controlling disturbances in cases in which an inferior nervous make-up is the dominating factor. On the other hand, there is no doubt that very profound nervous distubances, and occasionally psychoses, may result from ovarian insufficiency during the active sex period of life.

A fact which is to be considered in the endocrine treatment of these patients is the possible necessity of utilizing some other preparation to supplement ovarian treatment. This applies especially to the pituitary and thyroid glands. There is a growing recognition of the importance of the pituitary function in its relation to gynecological conditions. Absence or scantiness of the menses, accompanied by a rapid increase in weight, polyuria, and marked carbohydrate tolerance, points strongly to a pituitary insufficiency. Undoubtedly the unsatisfactory results sometimes experienced in the glandular treatment of these cases are attributable to the failure

of the physician to recognize these intercurrent factors. The intelligent application of glandular treatment depends upon the ability to interpret the cardinal symptoms of the important glandular aberrations.

A number of clinical reports have been made from time to time with regard to the use of ovarian preparations in the treatment of dysmenorrhea. It is difficult to understand why such therapy should prove beneficial. Undoubtedly the majority of cases of dysmenorrhea are attributable to anatomical lesions or defects of the uterus or its adnexa. Faulty development of the internal genitalia accounts for a considerable percentage. However, looking at the question from a standpoint of glandular function, we would naturally expect a hyperfunction of the ovaries rather than the converse; in which event ovarian treatment would be distinctly contraindicated. On the other hand, it has been suggested that in some cases dysmenorrhea is due to a hypersensitive nervous system, and that ovarian deficiency predisposes to such a condition. Some favorable clinical reports have been submitted to support this idea. It must be conceded, however, that the efficacy of ovarian treatment in dysmenorrhea has not been well substantiated, and that the usefulness of such treatment is to be largely limited to the early puberal period as an agent to promote the development of the internal genitalia.

Menorrhagia, in the majority of cases, is undoubtedly attributable to organic conditions, but there is a certain group associated with excessive ovarian activity. Here again ovarian or corpora lutea treatment would be distinctly contraindicated. It is an interesting fact that mammary preparations appear to be useful in these conditions. That the mammary gland secretes a substance which has a neutralizing effect on the ovarian secretion is fairly well substantiated. The abatement of the menses during gestation and the rarity of impregnation during the period of lactation are illustrations of the relation existing between these two glands. The therapeutic side of the question has not been exhaustively studied, but such clinical evidence as is available indicates that the mammary exerts a definite effect in neutralizing ovarian hypersecretion.

These passing references to a few practical applications of glandular treatment, not universally understood, are merely illustrative. Endocrine therapy does offer the therapeutic possibilities of un-

doubted importance. Its real usefulness will not be realized, nor its limitations appreciated, until medical men have abandoned the "cut-and-try method," and apply such treatment, guided by intelligent interpretation of the various glandular syndromes.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 228, 1919.

(Reprinted from the *American Journal of Pharmacy,* Vol. 91, No. 10, October, 1919.)

CHLORETONE: TRICHLORO-TERTIARY-BUTYL ALCOHOL. A DESCRIPTION OF SOME OF ITS PROPERTIES.

By Herbert C. Hamilton.

(From the Medical Research Laboratories, Parke, Davis & Co., Detroit, Mich.)

This is a compound formed by the direct union of chloroform and acetone, a reaction which is initiated by a caustic alkali. Willgerodt[1] discovered the reaction in 1881 and produced the compound which he called acetone-chloroform. When purified by steam distillation, or when recrystallized from water, it melts at 80°-81° C., somewhat higher when freed from water by distillation.

Chemical Properties.—The empiric formula for chloretone shows it to be apparently a direct combination of chloroform and acetone. Its structural formula, however, indicates that the compound takes on the formation of an alcohol and thus accounts for the chemical designation of trichloro-tertiary-butyl alcohol. It is soluble in most organic solvents and oils and is soluble in water—about 0.8 per cent.—from which it crystallizes in slender, white needles.

Physiological Studies.—The physiological actions of this compound have been studied by Abel and Aldrich,[2] Kossa,[3] Vamossy,[4,5] Houghton and Aldrich,[6,7] demonstrating its action as a local and general anesthetic and as a hypnotic and sedative.

As a General Anesthetic.—It has become the general anesthetic of choice for work on laboratory animals, its exceptional value depending upon the fact that it is safe and relatively non-toxic, and that one dose is sufficient to maintain complete anesthesia for several hours uncomplicated by any serious effects on the heart and circulatory system. This applies only to such experimental work

as involves an examination of the pharmacologic properties of drug or gland extracts, for example, the standardization of extracts of the suprarenal and pituitary glands where the effect is to raise the blood pressure, study of the digitalis series of heart tonics which affect primarily the circulatory system, of aconite and veratrum which are circulatory depressants, of blood coagulants which act to decrease the time of blood clotting.

For operations in which the recovery of the patient is of first importance, chloretone can be used in conjunction with morphine by which complete anesthesia can be accomplished, using a sublethal dose of the chloretone, but a dose large enough to prolong the anesthesia over a period of several hours. The technic for such work has been well described by Rowe,[8] for while there is no difficulty involved, attempts to apply this method of anesthesia have not always been successful.

As a Hypnotic and Sedative.—Another very common use made of chloretone is to allay the nausea due to seasickness. This is probably brought about, not only by the sedative and anesthetic action of the drug on the stomach lining, but also by the general action on the central nervous system. Autopsies show that more chloretone is found in the brain than in any other organ of the body, which is a logical finding in view of its exceptional efficiency as a general anesthetic. While it is a highly volatile product, it appears not to be eliminated by the lungs nor as such in the urine, but is finally decomposed as shown by an increase in the chlorides. Chloretone is regarded by the medical profession as producing the closest approximation to natural sleep that has yet been discovered, in its safety and reliability and in the fact that no unpleasant after-effects are experienced. The substance is carried to the cerebral tissue and profound sleep occurs. After a time as the chloretone is gradually broken up and carried away chemical activity is renewed in the brain cells and the patient awakes, refreshed as from natural sleep.

The mode of administration seems to have little influence on its absorption, for animals kept in an atmosphere saturated with vaporized chloretone are anesthetized, in time almost as completely as if it were administered internally.

Insecticidal Action.—This action of the vapor suggested its use as a substitute for naphthalene as an insecticide for clothes moths.

Experiments were carried out on moths, flies and mosquitoes, which showed that for the latter insect chloretone is four times as effective as sulphur fumes and almost as effective as the latter for moths and flies. It is as effective for moths as naphthalene without the objectionable odor of the latter.[9]

In these experiments weighed quantities of the substance were vaporized in a bell jar or in a laboratory hood of known capacity and the condition of the insects or animals carefully noted. It requires four or five hours to anesthetize guinea-pigs, and it is necessary to volatilize the chloretone slowly, carrying the vapors in with a current of air. For insects which require less air the rapid volatilization in a short time is somewhat more effective than the slower method because of its prompt action.

As a Local Anesthetic.—When chloretone is tested by some of the laboratory methods used for comparing local anesthetics it is found to be surprisingly effective. Tested on the sciatic nerve of the frog, one of the standard methods applied for substances of this character and compared to cocaine the results are as follows:

Chloretone.

	0 Min.	5 Min.	10 Min.	15 Min.	20 Min.
0.8 per cent. solution.........	—	±	+	+	+
0.4 per cent. solution.........	—	—	+	+	±
0.2 per cent. solution.........	—	—	—	±	±

Cocaine.

	0 Min.	5 Min.	10 Min.	15 Min.	20 Min.
1 per cent. solution	—	±	+		
0.5 per cent. solution.........	—	±	+		

Another test applied to local anesthetics is to measure the anesthetic action on the frog's skin. This is always moist and is comparable to the mucous membrane. The test is carried out by dipping one leg of the frog into the solution to be tested, leaving the other undipped as a normal for control. After contact for a determined time both legs are dipped into a very dilute solution of HCl—about 1-500. Anesthesia can be measured by finding the maximum dilution which is effective and comparing with a solution of cocaine of equal activity. The following results were obtained:

Chloretone.

Minutes.	0.8 Per Cent. Solution.	0.4 Per Cent. Solution.	0.2 Per Cent. Solution.
0	—	—	—
2	+	+	—
5	+	+	±
8	+	+	—
10	+	±	—
15	+	—	—

Cocaine.

Minutes.	1 Per Cent. Solution.	0.5 Per Cent. Solution.	0.25 Per Cent. Solution.
0	—	—	—
2	—	—	—
5	±	±	—
8	±	±	±
10	+	+	±
15	+	±	—

Note.—Minus, —, no anesthesia. Plus, +, complete anesthesia. Plus minus, ±, partial anesthesia.

As an anesthetic for the mucous membrane, therefore, it is even better than cocaine, and in its direct action on the exposed nerve, as on the sciatic nerve, the anesthesia is as prompt and as lasting as that of cocaine.

It fails, however, to replace cocaine because of its low solubility. It is precipitated in the tissues and is rather irritating and ineffective on that account. Its local action on the sense of taste is shown by the fact that its rather bitter disagreeable taste is only momentary in the mouth, but when the solution reaches the throat the objectionable taste is again evident.

Aside from the wide applicability of chloretone as a general anesthetic, its most valuable property is as a germicide and antiseptic, the properties essential in a preservative. If the "bone-dry" legislation keeps on, chloretone will be one of the few preservatives left for organic medicinals.

Germicidal.—As a germicide it has a phenol coefficient of 1.2—that is, when tested by the Hygienic Laboratory method of evaluating disinfectants it is as effective in 0.8 per cent. solution or 1-120, as phenol diluted 1 in 100; that is, a culture of *B. typhosus* is killed when exposed to the action of either of these two disinfectants for two minutes.

Tests by the A. P. H. A. Phenol Coefficient method[10] are given for chloretone and phenol.

GERMICIDAL EXPERIMENTS.

Typical Test of Phenol.

Dilution.	Time and Results, Minutes,			
	5.	10.	15.	20.
1–100	—	—	—	—
1–110	+	—	—	—
1–120	+	+	—	—
1–130	+	+	+	—
1–140	+	+	+	+

Test of Chloretone.

Saturated Aqueous Solution. (*B. Typhosus.*)

Dilutions.	Time and Results, Minutes.			
	5.	10.	15.	20.
5 cc. 0.8 per cent. sol. + 0 cc. water	—	—	—	—
9 cc. 0.8 per cent. sol. + 1 cc. water	+	+	—	—
8 cc. 0.8 per cent. sol. + 2 cc. water	+	+	+	+
7 cc. 0.8 per cent. sol. + 3 cc. water	+	+	+	+
6 cc. 0.8 per cent. sol. + 4 cc. water	+	+	+	+

+ means growth.

— means no growth in subculture.

Staphylococcus.

	5 Min.	10 Min.	15 Min.	20 Min.
0.8 per cent. solution	+	+	+	+

Spores of Hay Bacillus. (*B. Subtilis.*)

	1 Hr.	2 Hr.	3 Hr.	4 Hr.
0.8 per cent. solution	+	+	+	+

Mould Spores.

	1 Hr.	2 Hr.	3 Hr.	4 Hr.
0.8 per cent. solution	+	+	+	+

For an antiseptic test the two latter organisms were inoculated in medium saturated with chloretone. In no case was growth observed over a period of three weeks. Tested against other more resistant organisms it is found that in most cases to be effective it requires a longer time than a disinfectant can be expected to act, and therefore that it must be classed as a preservative or antiseptic rather than a germicide. For this purpose, with but few excep-

tions, it is ideal; even the highly resistant hay bacillus, *B. subtilis*, fails to develop in a solution saturated with chloretone. In some instances where the solution is an exceptionally good medium for the growth of bacteria it requires more than 4 days to become sterile, but evidence of growth is not apparent. It is absolutely essential that the container be closed against the volatilization of the chloretone, and a full container is advisable because of the tendency of chloretone to crystallize on the walls above the solution. This may be due to supersaturation, but if so it is a condition to be retained if possible. Such a solution can be obtained by adding the chloretone in a saturated alcoholic solution, by heating the chloretone in water, or by allowing several days for complete saturation.

As a preservative against mould spores it has not proved entirely satisfactory for serums and heavy organic solutions. The strains used in this experimental work have, however, invariably failed to develop in an agar-bouillon medium, but in practice moulds occasionally appear showing either loss of chloretone through volatilization or the presence of a more resistant variety. It seems probable that the occasional development of mould in pharmaceutical preparations preserved with this agent is due to a deficiency of the agent rather than a more highly resistant mould because of failure to obtain a saturated solution or a decrease in the chloretone content from other causes.

It is the purpose of this paper to emphasize two of the properties of chloretone which seem to be exceptionally valuable and which should commend it to laboratory purposes. First, as a general anesthetic for animal experimentation, it has no equal because of its long-continued action and non-interference with the circulatory system. Second, as a preservative where its antiseptic and germicidal action can be relied upon to prevent the development of bacteria and ultimately to kill the organisms which not only impair the appearance, but also destroy the valuable properties of organic solutions. It can be used in many cases where the preservative action of alcohol must be eliminated, and especially where sterilization by heat is impracticable because of its destructive action on sensitive organic compounds.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 229, 1919.

(Reprinted from the *Journal of the American Veterinary Medical Association,* Vol. LVI, N. S. Vol. 9, No. 1, October, 1919.)

STUDIES ON ANTHELMINTICS.

IV. Experiments with Combinations of Oil of Chenopodium and Chloroform.

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Whether a combination of oil of chenopodium and chloroform might be a valuable one, has been discussed by Hall and Foster. They state:

"Various writers have advocated the use of chloroform in castor oil in connection with oil of chenopodium or other anthelmintics, and some have claimed a synergistic action for chloroform and chenopodium. We have not found any convincing evidence of synergistic action. Chenopodium has a certain anthelmintic value for hookworms and chloroform has a greater value; but we have not observed that the simultaneous use of the two drugs adds to the efficiency of the chloroform alone. However, the combination of chenopodium and chloroform is a valuable one, as the chenopodium can be expected to remove ascarids, when these are present in hookworm cases, and ascarids are commonly present in such cases."

The experiments supporting the above comment on the combination of chenopodium and chloroform were later published by Hall and Foster as follows:

In one experiment, chenopodium was given in the dose of 0.1 mil per kilo, the single therapeutic dose, daily for a total of 6 doses. It was mixed with 10 times its volume of olive oil and preceded by castor oil in amount equal to the olive oil; 1 minim of chloroform per kilo body weight was added to the olive oil-chenopodium mixture the first day and to the castor oil the fol-

*Resigned March 27, 1919.

lowing days. This treatment removed 8 out of 8 ascarids (100 per cent), and 94 out of 133 hookworms (71 per cent).

In a second experiment, 4 dogs were each given chenopodium and chloroform, each at the rate of 0.1 mil per kilo; the drugs were given in castor oil, from 12 to 40 mils, according to the weight of the dog. This treatment removed 7 out of 8 ascarids (87.5 per cent), and only 7 out of 61 hookworms (11 per cent).

In a third experiment, the dose of each drug was doubled, being given at the rate of 0.2 mil per kilo, and in about 30 mils of castor oil. This treatment removed 100 per cent of the ascarids and over half of the hookworms.

Hall and Foster state that 0.1 mil per kilo of each of these drugs is too small a dose for the best results. It is the opinion of Hall, as stated elsewhere, that 0.1 mil per kilo is the proper dose of oil of chenopodium for use against ascarids, representing a dose which will seldom fall below 100 per cent efficacy, as smaller doses do too frequently, and which is naturally less toxic than larger doses. As for chloroform, the dose of this drug for dogs should certainly be at least 0.2 mil per kilo, and 0.3 mil per kilo is well tolerated.

The writer is now of the opinion that under suitable conditions of administration, a single dose of both drugs will secure better results than a single dose of either one alone.

The following experiments indicate that a single dose of 0.1 m. p. k. of chenopodium and 0.2 m. p. k. of chloroform gives very satisfactory results where the two drugs are both used in the same treatment and the chenopodium is given in the soft, or soluble elastic, capsule. In these experiments, the dogs were given oil of chenopodium at approximately the rate of 0.1 m. p. k., so far as the dose could be approximated in the administration of soluble elastic 5- and 10-minim capsules, and were given chloroform at the rate of 0.2 m. p. k. in hard gelatin capsules, No. 00. The purgative, for which the kind and amount is specified, was given immediately after the other drugs. From what the writer has observed of the action of these drugs under these conditions, the chloroform would be very promptly released in the stomach; such purgatives as castor oil would rather promptly begin to pass out with their chloroform content, while such oils as olive oil would tend to remain in the stomach a rather long period; and the soluble

elastic capsules, when given with castor oil, would digest or open in the stomach within a half-hour or so. The castor oil in the stomach and its persistent purgative action in the intestine would carry along the chenopodium, whenever it left the stomach.

Dog No.	Wt.—Kilos.	Chen.—Mms.	Chlor.—m.p.k.	Purgative	Efficacy (%) Against Ascaris	Hookworms	Whipworms	Tapeworms	Digestive Tract
23	11	15	0.2	Ol. ric. 30 mils.	....	100	...	....	Normal
27	12	15	"	Ol. ric. 30 mils.	..	75	0	100	Normal
28	17	25	"	Ol. ric. 30 mils.	....	100	...	....	Normal
30	9	15	"	Ol. ric. 30 mils.	100	..	..	....	Normal
31	6	10	"	Ol. olivae 60 mils. sod. sulph., 2dr.	...	...	0	0	Gast.-ent. hem.
32	7	10	"	Ol. olivae 30 mils.	100	....	...	....	Gast.-ent. hem.
33	8	10	"	Ol. olivae 30 mils. calomel 2gr.	100	57	...	0	Ent. hem.
34	9	10	"	Ol. olivae 30 mils. 2 C. C. pills	40	....	....	...	Ent. hem.
35	8.5	10	"	Ol. olivae 30 mils. Elaterin gr. 0.1	83	....	....	0	Normal
38	10	15	"	Ol. lini 60 mils.	100	92	....	....	Ent. Infl.(mod.)
40	10	15	"	Ol. ric. 60 mils.	100	100	....	....	Normal
41	10	15	"	Ol. ric. 60 mils.	No conclusions. Sickly animal; died next day.				
60	7	10	" †20	Ol. ric. 20 mils. Ol. olivae 20 mils.	....	....	0	0	Inflam.
64	9	15	0.2	Ol. coco. 30 mils.	100	....	0	....	Inflam.
67	8	15	"	Ol. ric. 60 mils. in S. E. capsules.	100	....	100	0	Inflam.
70	9	15	"	Ol. ric. 20 mils. Ol. olivae 20 mils.	....	....	0	0	Hem.

†Minims.

There are 16 dogs in the foregoing table, of which one, No. 41, must be disregarded in drawing conclusions, as the animal, a clinical case of uncinariasis, died the day following treatment, too early to permit of anthelmintic action. The other 15 dogs had a total of 150 ascarids, an average of 10 per dog; 40 hookworms, an average of 5.6 per dog. Of these worms, the combined chenopodium (0.1 m. p. k.) and chloroform (0.2 m. p. k.) treatment removed 146 ascarids (97 per cent), 35 hookworms (87.5 per cent), 4 whipworms (12.5 per cent), and 1 tapeworm (1 per cent). These percentages for efficacy against ascarids and hookworms are high. The showing against hookworms is especially high for single dose treatments and is better than the average obtained by single doses of either drug alone. It is better than

the result obtained by Hall and Foster in giving both drugs at the rate of 0.2 m. p. k. in 30 mils of castor oil. This result can hardly be regarded as a true case of synergistic action, something Hall and Foster have regarded as not present in the anthelmintic action of these drugs, but it is possible that treatments given in this fashion do allow one drug to supplement the other. As was noted already, the chloroform probably passes down the digestive tract promptly, mixed with the purgative, and is followed shortly afterward by the chenopodium. It is quite possible that worms that are not removed by the chloroform, but are weakened by a sub-lethal dose, succumb to the additional toxic effects of the chenopodium to which they are later subjected. Or it may be that some hookworms not killed by the chloroform are deprived of their fatty protective coating by the chloroform and so exposed to attack by chenopodium, or loosen their attachment to the mucosa, under the influence of the chloroform, and are caught in the lumen of the gut by the chenopodium. Unfortunately, we have only speculation on this subject at present. That the combination was efficacious here, however, is not a matter of speculation.

A consideration of the condition of the digestive tract in these animals shows the following: Leaving out of consideration Dog 41, which was too sick to tolerate treatment and cannot be regarded as a normal animal, 6 dogs had normal digestive tracts, 3 had inflamed digestive tracts, and 6 had hemorrhagic digestive tracts. Since the anthelmintic treatment was the same for all, the reason these findings may be sought in the purgation employed, since the eliminative and distributive action of such drugs as castor oil has been found protective by the writer against lethal doses of such drugs as chenopodium or oleoresin of male-fern, whereas the tendency of olive oil to remain in the stomach and be absorbed has been found to result in serious damage to that organ. These findings, published elsewhere by the writer, are confirmed by the findings here. Where castor oil alone was used, the digestive tract was normal five times and inflamed once; where olive oil was used, alone or with a diverse assortment of purgatives, the digestive tract was hemorrhagic six times and normal once; where other oils, cocoanut or linseed, were used, the digestive tract was inflamed twice. These findings confirm the desirability of using castor oil as a purgative with chenopodium, something which a

number of writers have advised, though some have given a preference to Epsom salts. It is likely that either is satisfactory; the important thing is prompt and adequate purgation.

In the following experiments, the dose of chenopodium was cut to half the therapeutic dose of 0.1 m. p. k., or 0.05 m. p. k., the dose of chloroform being the same as in the preceding experiments.

Dog No	Wt.—Kilos.	Chen.—Mms.	Chlor.—m p k	Purgative	Efficacy (%) Against				Digestive Tract
					Ascaris	Hookworms	Whipworms	Tapeworms	
39	6	5	0.2	Ol. ric. 30 mils...............	100	75	....	...	Normal
42	11	10	"	Ol. ric. 60 mils...............	75	83	0	....	Ent. hem.
43	8	5	"	Ol. ric 60 mils...............	100	....	0	100	Normal

In this diminished chenopodium dosage, the treatment removed 8 of a total of 9 ascarids (an efficacy of 89 per cent); 8 of 10 hookworms (80 per cent); none of 11 whipworms (0 per cent); and both of 2 tapeworms (100 per cent). While this is a good showing for ascarids, numerous experiments indicate that this smaller dose of chenopodium, 0.05 m. p. k., will fail in a distinctly larger number of cases than the larger dose of 0.1 m. p. k., and as this latter is well tolerated, it should be used. The efficacy of the treatment against hookworms is unimpaired, a showing in keeping with the undiminished chloroform dosage of 0.2 m. p. k.

The digestive tract was normal in 2 of the 3 dogs, and showed some enteric hemorrhage in the third. Castor oil, in 30 and 60 mil doses, was used.

One dog, No. 47, was given the therapeutic dose of chenopodium, 0.1 m. p. k., and a diminished dose of chloroform, 1.33 m. p. k. This dog weighed 15 kilos, received 20 minims of chenopodium, 2 mils of chloroform, and 60 mils of castor oil. It passed 6 ascarids and had 3 whipworms post-mortem, an efficacy of 100 per cent against ascarids and 0 per cent against whipworms. There were some inflamed areas and some hemorrhage in the intestine.

In another series, the dogs were given double the therapeutic

dose of chenopodium, or 0.2 m. p. k., with a therapeutic dose of chloroform, 0.2 m. p. k.

Dog No.	Wt.—Kilos.	Chen.—Mms.	Chlor. m. p. k.	Purgative	Efficacy (%) Against				Digestive Tract
					Ascaris	Hook-worms	Whip-worms	Tape-worms	
49	14	40	0.2	Ol. gossyp. 60 mils.	100	....	....	....	Ent. inflam.; pyloric hem.
50	12	35	"	Ol. ric. 30 mils.	100	....	0	0	Gast.-ent. hem.
51	6	15	"	Ol. sesam. 60 mils.	100	0	....	....	Ent. hem.
52	6	15	"	Ol. arach. 60 mils.	100	....	0	0	Gast. hem.

With this double dosage of chenopodium, the treatment removed all of the 35 ascarids present (100 per cent), and none of the other worms, including 1 hookworm, 10 whipworms, and 24 tapeworms (0 per cent). In the experiments already noted, chenopodium in doses of 0.1 m. p. k. and 0.05 m. p. k. removed 160 ascarids and failed to remove 5. While it might be thought that this established the larger dosage as the preferred one, it really does not do so. Experiments published by Hall and Foster show that chenopodium, in single doses of 3 m. p. k. or 3 doses of 0.2 m. p. k. each on successive days, will fail to remove occasional ascarids, and these failures are to be attributed, in the present state of our knowledge, to conditions of which we are unaware and over which we have at present no control. No safe dose of any anthelmintic known can be depended on to remove all worms present every time. This fact, coupled with the fact that anthelmintics are toxic substances which should be used with caution, makes the employment of the 0.1 m. p. k. dose of chenopodium advisable. The occasional deaths of weak animals, following the administration of conservative therapeutic doses of anthelmintics, inclines one to avoid larger doses than are necessary for reasonably dependable results, and these results can be obtained against ascarids with the 0.1 m. p. k. dose of chenopodium.

These single doses of chenopodium and chloroform removed a total of 43 out of 51 hookworms, an efficacy of 84 per cent. This is a very good showing against a worm that is difficult to

remove, and these two drugs, administered in the manner noted, give results superior to those obtained as a rule with either drug alone or with drugs other than these.

The failure of most treatments in these experiments to remove whipworms or tapeworms is to be expected. The drugs and the mode of administration are not appropriate for these worms.

The following experiments are intended to determine the efficacy of *repeated* doses of chenopodium and chloroform.

Dog No. 46, weighing 25 kilos, was given 20 minims of chenopodium in the soluble elastic capsule, with 2 mils of chloroform in hard capsules and 60 mils of castor oil. Six days later the dog was given 75 minims of chenopodium in soluble elastic capsules, with 5 mils of chloroform in 60 mils of castor oil, and six days later the dog was given this same dose again. Five days later the dog was killed. No worms were passed and 5 whipworms were found post-mortem. Even such rather large doses, repeated at these intervals, were 0 per cent effective against whipworms, which is not surprising in view of the location of these worms.

In the following experiments, the dogs were given 3 doses of oil of chenopodium in soft, or soluble elastic, capsules, each dose consisting of the amount shown in the table and being given with 15 mils of castor oil or without the oil, as indicated. The last dose was followed after an interval of 1 to 1½ hours by 4 mils of chloroform in 15 mils of castor oil if the chenopodium was given with oil each time, and 30 mils if no oil had been given. These experiments, briefly tabulated here, were published in full by Hall (1919).

Dog No.	Wt.—Kilos.	Chen.—Mms.	With ol. ric.	Efficacy (%) Against				Digestive Tract.
				Ascaris	Hook-worms	Whip-worms	Tape-worms	
289	21	10	Yes..........	100	86	.....	.....	Gast.-Ent. hem.
301	15	10	Yes..........	100	97	....	...	Gast. inflam., Ent. hem.
300	18	5	No..........	......	50	....	0	Few ent. petechiae.
292	14.5	5	Yes..........	....	97	. .	0	Gast. inflam.

From an anthelmintic standpoint, this treatment was decidedly successful. It removed 6 out of 6 ascarids (100 per cent) and

127 out of 142 hookworms (89 per cent). In the 3 cases where the capsules were given with castor oil, the treatment was 91 per cent effective against hookworms. There were no whipworms present, and it failed to remove any of 17 tapeworms, a matter of no moment, as it is not a tæniafuge anyway.

As regards the effect on the host, it was especially bad in the case of Dog No. 301, but this dog had distemper when treated and died on the third day after treatment. The writer has pointed out elsewhere that dogs having distemper should not be given anthelmintic treatment until after they have recovered. While there was a production of inflamed and even hemorrhagic areas in the mucosa of the stomach and intestines, the condition was not serious and would have cleared up in the course of 10 days or two weeks. Compared with the damage done by hookworms, this is something that may be tolerated, and in view of the high degree of efficacy attained, the treatment outlined here would be the one indicated. It will be noted that where castor oil was not given with each dose of chenopodium, the efficacy fell to 50 per cent. The writer prefers the simultaneous administration of castor oil with the capsules. Our experiments show that the capsules given with castor oil will open in a half-hour or so, so that the worms are first subjected to chenopodium and later to chloroform.

In the following experiments, dogs were given soluble elastic capsules each containing 5 minims of chenopodium and 10 minims of chloroform. The first series given will consider those dogs, 4 in number, which received 1 dose of these capsules, and the second series will consider those dogs, 11 in number, which received repeated doses.

The following dogs received these capsules to the number stated, followed immediately by 30 mils of castor oil.

Dog No.	Wt. Kilos.	Capsules	Efficacy (%) Against				Digestive Tract.
			Ascaris	Hookworms	Whipworms	Tapeworms	
95	14	4	......	....	0	....	Inflam.
96	17	4	....	0	0	0	Normal
97	9	2	......	....	0	0	Pyloric hem.
98	12	1	......	53	......	0	Gastric hem.

There were no ascarids present in these dogs. The treatment removed 8 out of 19 hookworms, an efficacy of 42 per cent. It failed to remove any of 9 whipworms and 11 tapeworms, an efficacy of 0 per cent against these worms. The digestive tract was normal once, inflamed once, and there were hemorrhages in the stomach twice.

Apparently the combination of chenopodium and chloroform in the soft capsule is not as effective as the administration of chenopodium in the soft capsule, with the practically simultaneous administration of chloroform in the hard capsule or dissolved in the accompanying castor oil, or with the chloroform administered after an interval. Furthermore, the effect on the stomach suggests that where these two drugs are thoroughly dissolved in one another, the chloroform may hasten the local absorption of the chenopodium with bad results. It is probable, from other experiments, that the castor oil that might serve as a protection to the stomach against this combination has passed out before this capsule opens. Probably the chloroform exerts a hardening effect on the gelatine. At any rate, the *simultaneous* administration of chenopodium and chloroform in this manner gives inferior results as regards worms removed and effect on the digestive tract, when compared with their practically consecutive administration as attained by the use of the soft capsules for the chenopodium only.

The following series comprises dogs receiving repeated doses of the soft capsules containing 5 minims of chenopodium and 10 minims of chloroform. All dogs received 1 capsule at a dose and 30 mils of castor oil with each dose.

Dog No.	Wt. Kilos.	Doses	Intervals	Efficacy (%) Against				Digestive Tract
				Ascaris	Hook-worms	Whip-worms	Tape-worms	
245	13	2	3 days..........	100	25	.....	....	Normal
243	10.5	2	4 days	....	100	.....	....	Normal
238	9	3	4 & 3 ays.......	100	42	90	.....	Gast.-Ent. hem.
239	10.5	5	4, 3, 4 & 2 days..	......	75	79	100	Gast.-Ent. hem.
230	8.5	5	4, 6, 5 & 2 days..	100	89	.....	......	Gast. hem.
183	11.5	6	(In 8 days)......	100	.....	.....	0	Gast.-Ent. hem.
184	5.5	7	(In 8 days)......	....	.....	.....	....	Gast.-Ent. hem.
182	8	7	(In 8 days)......	100	.....	.....	....	Gast.-Ent. hem.
181	13	7	(In 8 days)......	......	100	100	....	Gast.-Ent. hem.
177	15	7	(In 8 days)......	......	.....	100	.....	Gast. hem.
175	19	7	(In 8 days)......	...	100	.	.	Gast. hem.

These treatments removed 56 out of 56 ascarids (100 per cent); 45 out of 64 hookworms (70 per cent); 50 out of 59 whipworms (85 per cent); and 1 out of 2 tapeworms (50 per cent). The digestive tract was normal twice, following 2 doses, and showed gastric hemorrhage in the other 9 dogs, following 3 to 7 doses, coupled with enteric hemorrhage in 6 of these cases.

A consideration of the above table and summary indicates that repeated doses of soft capsules containing 5 minims of chenopodium and 10 minims of chloroform are entirely efficacious against ascarids, as would be expected. They are only 70 per cent effective against hookworms, a high efficacy compared with chenopodium or chloroform alone in single doses, but not so high as the efficacy (87.5 per cent) attained by single dose treatments where oil of chenopodium is given at the rate of 0.1 m. p. k. in soft capsules, and chloroform is simultaneously given at the rate of 0.2 m. p. k. in hard capsules with 30 mils of castor oil or dissolved in the castor oil, or the efficacy (89 per cent) of 3 doses of chenopodium at hour intervals followed by chloroform 1 to 1½ hours later. It may be noted that in these repeated doses the individual dose was less than the therapeutic dose of 0.1 m. p. k., as it must be in repeated doses. At the same time, the condition of the digestive tract indicates that this combination of drugs is not well suited to repeated doses, being too irritant. The high efficacy against whipworms (85 per cent) is to be expected, as the keynote to treatment for infestation with this worm is repetition, a fact that the writer has pointed out in previous articles. The irritant character of chenopodium and chloroform makes them unsuitable for repeated doses and they are best used in single dose treatments or treatments calling only for repeating doses during the course of a few hours of one day. Santonin, on the other hand, has too little efficacy in single dose to be as valuable as chenopodium for ascarids and is of no value for hookworm treatment, but the fact that it does not act as a gastro-intestinal irritant makes it the drug of choice where whipworms are concerned, since it can be safely given in the repeated treatments which are necessary to ensure some of the drugs finally entering the cecum and removing the worms (safely, that is, so far as gastro-intestinal irritation is concerned).

Experiments with these soft capsules, containing 5 minims of chenopodium and 10 minims of chloroform, after they had been

enteric-coated by exposure to formalin, have been detailed in another paper. The results were unsatisfactory.

SUMMARY.

The best results in the removal of hookworms by a single dose treatment of which the writer is aware, were obtained by the use of oil of chenopodium in a dose approximating 0.1 m. p. k., as near as the dose can be approximated in 5- and 10-minim soft, or soluble elastic, capsules, followed immediately by 30 mils of castor oil containing chloroform at the rate of 0.2 m. p. k., or with this amount of chloroform given in hard gelatine capsules. Apparently the results so obtained are due to consecutive action which approximates treatment by repeated doses, as the chloroform in castor oil acts very promptly against the worms, and the chenopodium only after its release at some interval from the soft capsule. The treatment was 87.5 per cent effective in tests on 15 dogs, of which 6 had hookworms.

This same treatment was 97 per cent effective against ascarids in tests on 15 dogs, of which 9 had ascarids.

The treatment was comparatively ineffective against whipworms, which require repeated treatments, not single treatments.

The treatment was also ineffective against tapeworms, but neither of these drugs is dependable against tapeworms.

Very satisfactory results were also obtained by administering 3 doses of 5 or 10 minims of chenopodium in the soft capsule at hour intervals, accompanied each time by 15 mils of castor oil, and followed 1 to 1½ hours later by 4 mils of chloroform in 15 mils of castor oil. This treatment was 91 per cent effective against hookworms in tests on 3 infested dogs.

The same treatment was 100 per cent effective in tests on 2 ascarid-infested dogs and may be depended on to maintain substantially this efficacy.

These results bear out the claims of Schüffner and Verwoort for the treatment used by them in Sumatra. They gave 3 doses of oil of chenopodium, in 16-minim doses on sugar, at hour intervals, and 2 hours after the last dose gave 17 grams of castor oil and 3 grams of chloroform. Our experiments indicate that the patient is better protected when each dose of chenopodium is accompanied by castor oil, otherwise a treatment approximating treat-

ment used by Schüffner and Verwoort should be the best treatment for hookworms in man of those yet published, so far as our experiments indicate.

The simultaneous administration of chenopodium and chloroform, by giving a soft capsule containing both of these drugs in the proper proportion, is unsatisfactory. In single dose the combination does not display enough efficacy, and in repeated doses the efficacy is less against hookworm, the particular work for which this combination is intended, than the single dose using soft capsules for the chenopodium only. It shows the increased efficacy against whipworms that may be expected from repeated doses, but shows a corresponding degree of injury to the gastro-intestinal mucosa that makes it undesirable.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 230, 1919.

(Reprinted from the *Journal of the American Veterinary Medical Association,* Vol. LVI, N. S. Vol. 9, No. 2, November, 1919.)

STUDIES ON ANTHELMINTICS.

V. The Administration of Oil of Chenopodium in Soft, or Soluble Elastic, Gelatin Capsules, as Compared with Other Modes of Administration.

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Two of the very evident advantages afforded by the use of soft gelatin capsules are these: They furnish a convenient measured dose, and they supply a capsule with the advantages of the hard capsules (tastelessness) plus the advantage of saving bulk and bother, by combining the drug and the capsule, and the assurance that the capsule will not open in the mouth, pharynx or esophagus. They have proven entirely satisfactory in the administration of oil of chenopodium to experiment animals in our work here and have been recommended by one of us (Hall, 1917) for use in treating dogs.

The soft capsule, however, has recently been criticized as a container for oil of chenopodium, in a paper by Darling, Barber and Hacker (1918). They state that 3 10-minim doses of oil of chenopodium in soft capsules at hour intervals removed only 66.4 per cent of the hookworms from patients, as compared with the removal of 97.9 per cent of the worms by the same oil when removed from the capsule and then administered in freshly filled hard capsules. By way of explanation they state: "The soft capsules dissolved too slowly to permit their contents to affect the hookworms distributed in the duodenum and the upper part of the jejunum." Undoubtedly, this explanation is intended only as a plausible surmise. If the soft capsule digests or opens in the middle of the jejunum, it undoubtedly misses worms in the upper jejunum and duodenum, but there is some question as to whether it leaves the stomach unopened.

There are several ways of judging whether the soft capsule actually opens in the jejunum or in the stomach. One way is to determine the anthelmintic efficacy of chenopodium exhibited in the soft capsule and compare it with the efficacy of chenopodium exhibited in the hard capsules. Another way is to administer the soft capsules to dogs and kill the dogs after various intervals. We have here data of both sorts, and these data do not sustain the attitude taken by the writers quoted. So far as the application of findings in animal experiments to similar conditions in man is concerned, one must undoubtedly make the application with much care and some reservations. It is therefore impossible to insist too strongly that what we find true in the case of dogs is also true in the case of man, especially when the writers quoted find to the contrary. At the same time there is something to be said for our experimental findings.

In the first place, our experimental findings are exact and definite, covering treatment, exact number of worms passed for the 4 or more days after the administration of the anthelmintic and up to the day of death of the animal, and the exact number of worms found post-mortem. There can be no question but what the information obtained in this way is more exact than that obtained clinically and by examining the feces for parasite eggs, which method must be depended on by physicians working with human patients.

In the second place, hundreds of experiments show that the ascarid of the dog has a quite definite and dependable reaction to chenopodium, as Hall (1918) has noted, whereas hookworms in man or dogs have no such dependable reaction to any drugs, part of them yielding to one treatment and part quite commonly yielding only after 2 to 5 or more treatments. In judging, therefore, the fate of soft capsules of chenopodium, their results in the shape of ascarids removed from the dogs is a surer guide than that in the shape of hookworms removed from man and subsequent fecal examinations for eggs.

Finally, we have made a number of tests in the way of killing dogs at an interval after administering chenopodium in soft capsules and in other ways, and these tests show that the soft capsule does not wait until it reaches some point in the intestine before opening, but opens in the stomach. In this respect, our findings

agree with the fact that our critical tests show that soft capsules of chenopodium display as high anthelmintic efficacy against ascarids as do doses of the drug in hard capsules or in castor oil. We have data on 220 dogs that have been given oil of chenopodium or its derivatives, alone or in combination with other anthelmintics, in castor oil, hard or soft capsules, or undiluted and without capsule, with records of all worms passed and worms present postmortem, and these experiments all testify to the ascaricidal efficacy of chenopodium and bear out the statement that it is as effective when given in the soft capsule as when given in the hard capsule or any other way.

In this series of 220 dogs, there are only 8 that were given chenopodium in soft capsule, followed immediately with castor oil and not complicated by other considerations (enteric coats, other drugs, repeated doses involving more than 1 day, etc.), and these 8 dogs may be compared with the animals treated by Hall and Foster (1918). These writers gave chenopodium at the rate of 0.3 m. p. k. (mil per kilo) in castor oil to 8 dogs and removed 160 ascarids out of 162, an efficacy of about 99 per cent; they gave chenopodium in hard capsules at the rate of 0.2 m. p. k. with olive oil and castor oil on 3 successive days to 8 dogs and removed 15 out of 17 ascarids, an efficacy of 88 per cent. In our 8 experiment dogs, all of which were infested with ascarids, 4 dogs received the therapeutic dose of 0.1 m. p. k., 1 received a lethal dose of 1.0 m. p. k. with castor oil for protection, and 3 received doses between 0.1 and 0.2 m. p. k., all in soft capsules. These dogs passed 87 out of 87 ascarids, an efficacy of 100 per cent. In spite of the larger doses used by Hall and Foster, the efficacy secured by larger doses with their mode of administration is slightly inferior to that secured with smaller doses in soft capsules. They report that the dogs in the first series noted above had about one-fourth of their hookworms removed, and in the second series the treatment was ineffective for hookworm. In our series of dogs, 7 had hookworm; the treatment with soft capsules removed 23 out of 31, an efficacy of 74 per cent. In a series of 4 dogs treated by us with single doses of chenopodium, not in soft capsules, at the rate of 0.1 m. p. k. the treatment removed 10 of 10 ascarids and none of 16 hookworms from 2 dogs; at a higher rate it removed 5 of 5 ascarids from 2 dogs.

So far as anthelmintic efficacy is concerned, accurate critical tests on both ascarids, a dependable form for test, and hookworms, a less dependable form, show that if there is any choice between the administration of oil of chenopodium in soft capsules or hard capsules or in castor oil or olive oil, the choice would lie with the soft capsules.

As regards the time and place of opening of the soft capsule in the digestive tract, a series of experiments was performed to furnish information on this point. The first set of tests were with the soft capsules; the second set were with chenopodium administered in oil.

The first set of tests using the soft capsules may be summarized as follows:

Without purgative.

Dog No.	Capsules.	Dog killed.	Result, capsules.	Digestive tract.	Remarks.
251	3 5-minim	26 min.	In stomach; unopened.	Normal.	Food in stomach.
252	2 5-minim	42 min.	In stomach; unopened.	Normal.	Food in stomach.
253	3 5-minim	1 hr., 2 min.	In stomach, 1 opened, 1 cracked, 1 softened.	Normal.	Food in stomach.
254	3 5-minim	1 hr., 21 min.	In stomach, unopened.	Normal.	
354	3 5-minim	1 hr., 30 min.	Digested; chen. odor in stomach and small int.	Petechiæ in stomach and small int.	
348	3 5-minim	2 hrs.	Digested; chen. odor in stomach.	Normal.	Food in stomach.
331	3 5-minim	2 hrs., 46 min.	Digested.	Gas. int. hem.	

With purgative.
(These dogs all received 3 5-minim capsules and the purgative shown.)

Dog No.	Purgative.	Dog killed.	Result, capsules.	Digestive tract.	Remarks.
328	Ol. ric. 30 mils	30 min.	Digested; chen. odor and ol. ric. near ileocecal valve.	Mild hyperemia.	
355	Cascarin 2 grs.	30 min.	In stomach; open and half digested.	Gast. int. petechiæ.	
329	Ol. ric. 30 mils	1 hr.	Digested. Chen. and ol. ric. in stomach.	Normal.	
349	Calomel 2 grs.	1 hr.	Digested. Chen. odor in stomach.	Inflam. and hem.	Dog vomited fragments of capsule.
330	Ol. ric. 30 min	2 hrs., 4 min.	Digested. Oil in large int.	Normal.	1 asc. pres. in large int.

It will be noted from an examination of the above tables, that when the soft capsules were given *without purgatives,* they were recovered from the stomach unopened as late as 1 hour and 21

minutes after administration; they were found opened in the stomach as early as 1 hour and 2 minutes after administration; and they were entirely digested at intervals of 1 hour and 30 minutes to 2 hours and 16 minutes. It appears, then, that soft capsules given without purgation will open in the stomach, and probably open from 1 to 2 hours after administration. This coincides with the time of onset of symptoms of salivation and vomiting in animals given chenopodium in soft capsules, where these symptoms appear. Chenopodium could be found in the small intestine as early as 1 hour and 30 minutes after administration.

Where the soft capsules were given *with purgatives*, the time of opening in the stomach was materially shortened. As early as a half-hour after administration, and after all longer periods, the capsules were partly or entirely digested, and chenopodium was detected near the ileocecal valve in 30 minutes in one instance.

It will be noted that in no cases were capsules found unopened in the intestine. When they were found at all, they were in the stomach: here they were found unopened, open, and partly digested: here the oil of chenopodium could be detected in almost all cases, as late as an hour after administration in the case of capsules given with purgatives, and as late as 2 hours after administration in the case of capsules given without purgatives.

Not only does all the evidence point to the opening of the capsule in the stomach, but other considerations sustain this idea. The soft gelatin capsule becomes very much softened by exposure to the moisture of the digestive fluids of the stomach, not to mention the digesting action of these fluids. Such softened capsules break very easily on pressure, and it seems likely that the pressure applied at the pylorus would break these capsules if they started to pass unopened. Possibly the early opening of capsules given with purgatives is due to increased gastric peristalsis and prompt rupture of the softened capsule, with digestion of the capsule hastened as a result. It is also possible that the purgative stimulates secretion of the digestive fluids.

Following up the foregoing experiments, some tests were made to ascertain the fate of oil of chenopodium given without capsule of any sort, with and without purgatives. To aid in following the course of the chenopodium, it was mixed with Eaton's fat-soluble blue. The doses were at the rate of 0.1 m. p. k.

Without purgative.

Dog No.	Dog killed.	Oil found.	Digestive tract.	Remarks.
318	47 min.	In stomach.	Normal.	
321	1 hr., 4 min.	In stomach and small intestine.	Petechiæ in pyloric stomach; pylorus and duodenum hyperemic.	
319	1 hr., 45 min.	In stomach.	Normal.	

With purgation.

Dog No.	Dog killed.	Oil found.	Digestive tract.	Remarks.
320	32 min.	In stomach.	Normal.	30 mils ol. ric.
322	1 hr., 4 min.	In stomach.	Normal.	30 mils ol. ric.
351	1 hr., 30 min.	In stomach.	Gast. hem., small int. hyperemic.	2 grs. cascarin. 1 asc. in int., apparently dead.
357	2 hrs.	In stomach.	Normal.	2 grs. cascarin.
352	2 hrs., 7 min.	In stomach.	Few hem. in int.	2 grs. calomel. 2 asc. in int., apparently dead. Fed before dosing. Vomited in hr. after dosing.
323	This dog was given the chenopodium with oil-soluble blue and 30 mils of castor oil, and 29½ hours later the dose was repeated, this time with oil-soluble orange instead of blue. The dog was killed 2 hours and 12 minutes later. The orange-colored chenopodium was found in the ileum; there was no trace of the blue-colored chenopodium given the previous day. The ileum was mildly hyperemic.			

In view of the fact that small amounts of oil, such as would slowly pass the pylorus, cannot readily be detected even when the fat-soluble coloring matter is used, one cannot affirm much as to where chenopodium was not present; one can make affirmation only as to where it was present. From the experiments tabulated above, we find that chenopodium given without purgatives or the use of capsules may still be present in the stomach 1 hour and 45 minutes after administration; when given with purgatives, without the use of capsules, it may still be present in the stomach 2 hours and 7 minutes later. We also find that even without purgatives, the oil may be in the small intestine in 1 hour and 4 minutes, and with purgation it may be in the ileum 2 hours and 12 minutes after administration, and the evidence of an apparently dead ascarid indicates that it may be there in an hour and a half. Doubtless it is in the small intestine earlier, as the experiments with capsules show.

The fact that the chenopodium in soft capsules, given with castor oil, may rapidly get to the small intestine, is perhaps due to the fact that the castor oil can exert its action at the start, unimpaired by the constipating chenopodium in initiating peristalsis.

The slowness with which the oil leaves the stomach confirms the position taken by Hall (1918) to the effect that anthelmintics

at least some anthelmintics, probably do not need to be allowed "time to act" on the worms before purgatives are administered. In this connection, Hall states:

"It is so generally believed and stated that anthelmintics should be allowed time to take effect before any purgation is attempted, that it seems almost unsafe to dispute the proposition. Dock and Bass even explain the failure of remedies to act effectively as possibly due to 'the rapid carrying down of the thymol by peristalsis to below the location of the worms,' even in the absence of purgation. My own experiences have led me to fear more the absorption of the drug in the stomach before reaching the site of the worms. In over two years' experiment work, involving the treatment and post-mortem examination of over 250 dogs, the results seem to be a little better, if anything, where the anthelmintic and the purgative are administered simultaneously than where the anthelmintic is allowed to precede the purgative by an hour or longer. Such combinations as oil of chenopodium and castor oil, chloroform and castor oil, santonin and calomel, etc., seem to be as effective as the ingredients of the combinations administered separately and at intervals. It is well known, of course, that such anthelmintics as areca nut are themselves purgative. Even if it were true that anthelmintics are more effective if purgation is postponed, and it can be shown that the patient is safer where the purgation is given with the anthelmintic, would it not be good practice to repeat a safe treatment oftener, rather than to give a less safe treatment fewer times?"

We are unable to reconcile the differences in the results obtained by Darling, Barber and Hacker and those obtained by us, as regards the value of the soft gelatin capsule of chenopodium, but we have no adverse comment to make on their results. We merely state our own results and indicate that there is a disagreement. There might have been some differences in the capsules used by them and the capsules used by us, and it might be that the capsules harden with age, though we have no evidence of our own on this point. The soft capsule can be hardened with formalin to the point where it will not digest, and there are other substances which will have the same effect.

Our findings further disagree with theirs on a number of other points which can only be mentioned here. For one thing, they

found oil of chenopodium given in liquid petrolatum 88.7 per cent effective, while soft capsules were only 66.4 per cent effective, whereas we find that the use of liquid petrolatum cuts the anthelmintic efficacy, even against ascarids, to zero at times, while the soft capsules have an ascaricidal efficacy very close to 100 per cent. Experiments covering this point have been published by Hall (1918) in a study by Hall and Hamilton (1918). Darling, Barber and Hacker state that, "Chenopodium is relatively just as efficacious in removing *Ascaris*, *Clonorchis* and *Tænia*" as in removing hookworms. So far as the dog is concerned, hundreds of critical experiments show that chenopodium is unequaled for use against ascarids, and is a very inferior and undependable anthelmintic for use against *Tænia* and *Dipylidium*, removing them but rarely and showing no such efficacy as is shown by male fern or kamala. They prefer magnesium sulphate to castor oil as a purgative with chenopodium. This is a moot point which we will discuss in another paper.

CONCLUSIONS.

On the basis of many experiments with dogs, we feel safe in saying that the following is true of dogs and that these findings apply in part to man:

1. The soft or soluble elastic capsules of chenopodium open in the stomach and not in the small intestine.

2. The foregoing statement is sustained by the high ascaricidal efficacy of the soft capsules of chenopodium, as well as by their efficacy against hookworms.

3. The administration of purgatives at the same time as the soft capsules hastens the opening of the capsules. Without the purgatives, capsules lie in the stomach as long as 1 hour and 21 minutes, and apparently digest in 1 to 2 hours. With the purgatives, capsules are wholly or partly digested in 30 minutes. Without purgatives, chenopodium from the capsules may be detected in the intestines in 1 hour and 30 minutes. With purgatives, chenopodium may be detected in the intestines in 30 minutes.

4. The more rapid opening of capsules given with a purgative is probably due to the mechanical action of heightened peristalsis on the softened moist capsule and to increased secretion of digestive fluids as a result of stimulation by the purgative.

5. Given in soft capsules, chenopodium may stay in the stomach as long as 1 hour and 45 minutes if given without purgatives, or 2 hours and 7 minutes if given with purgatives; or may be detected in the small intestine in 1 hour and 45 minutes if given without purgatives, or in 1 hour and 30 minutes if given with purgatives. It is probably present in the small intestine earlier.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 231, 1919.

(Reprinted from the *American Journal of Veterinary Medicine,* Vol. 14, No. 11, November, 1919.)

HEMORRHAGIC SEPTICEMIA OF CATTLE; WITH SPECIAL REFERENCE TO STOCK-YARDS PNEUMONIA.*

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The disease which has been called stock-yards fever, stock-yards pneumonia, shipping fever of cattle, malignant catarrhal fever, etc., has made its appearance in various parts of Michigan during the past three years, and the disease as it occurs in this State in no way differs from the same disease as described in other States by a number of veterinarians during the past five years. In all of the outbreaks of the disease that I have seen in Michigan, the history, symptoms and lesions found at autopsy tally very well with the condition as observed previously in Minnesota, and described by the late Dr. S. H. Ward.[1]

It has been suggested that this disease is a form of hemorrhagic septicemia, and there appear to be several reasons why this view has been taken. As a matter of fact it appears that definite and conclusive proof, in the form of scientific evidence, is lacking to prove that the Bacterium bovisepticum is the primary etiological factor. In the first place, the hemorrhagic septicemia organism is reported to have been isolated from cases of the disease. Then we have the septic pleuro-pneumonia of calves, as described by Poels[2] over thirty years ago, and believed by him to have been caused by a member of the hemorrhagic septicemia group. This disease coincides in practically every essential detail with our so-called stock-yards pneumonia, from a pathological standpoint. Further, we have many favorable reports to the effect that the disease is being successfully prevented by immunizing exposed cattle with hemorrhagic septicemia vaccines and bacterins.

*Read before Illinois State Veterinary Medical Association, Champaign, Ill., July 8, 1919.

It is not my intention in this paper to deny the etiological relationship of the hemorrhagic septicemia organism to the disease in question. I will admit, however, that the doubt has existed at various times. The mere finding of the Bacterium bovisepticum in the blood or tissues at autopsy is not sufficient proof in itself that the organism is the primary infecting agent. Bacillus suipestifer is found quite frequently in cases of hog cholera, but we know that is is not the cause of the disease. The results obtained with hemorrhagic septicemia vaccines and bacterins are certainly suggestive, but it is so difficult to get accurate and reliable data on treated and untreated animals under identical conditions, that we must withhold our final verdict until such time as suitable evidence is available.

NATURE OF THE DISEASE.

First of all, it should be remembered that several forms of bovine hemorrhagic septicemia have been described; namely, the abdominal, pectoral, nervous or meningeal, and the cutaneous or exanthematous form. As implied by these terms, the disease differs, in its clinical manifestations, with the part of the body affected. The last named form of the disease is believed, by some veterinarians, to be identical with the so-called "mad itch." Stockyards fever or pneumonia is thought to be the pectoral form of hemorrhagic septicemia.

The disease in question seems almost invariably to be associated with a short residence in certain of our large public stockyards. Whether this is an essential factor in the disease, or only a coincidence, is open to question. Young cattle, stockers and feeders, that have come into these yards, stayed there a few days, been sold and shipped out again, frequently develop the disease shortly after arrival at their destination. In most cases only a part of the animals in the shipment develop the disease, sometimes 20 per cent, sometimes 50 per cent. It should not be stated, perhaps, that the disease is confined to young cattle of any special market class. It is possible that butcher cattle may be just as susceptible, but owing to the fact that these cattle are slaughtered shortly after their arrival in the yards, the disease does not have time to develop. There have been cases reported in older cows, milkers and springers, animals that have been bought up in the

stock-yards by scalpers or speculators, and held too long awaiting a favorable sale. This point has been brought out by Palmer,[3] who observed the disease in the South St. Paul stock-yards. That the disease has a rather definite period of incubation cannot be denied.

In most outbreaks of the disease the first trouble is noted in about a week or ten days after the cattle have left the stock-yards. Sometimes a few cases are noted earlier, and it is easily possible that such animals may have had an earlier exposure than the rest, may have been in the yards longer, or may have been more susceptible. Cases rarely develop later than fifteen days after shipment.

SYMPTOMS.

In some cases it would be very difficult, indeed, to differentiate between the disease under consideration and a case of ordinary pneumonia. However, with the history of having recently passed through a public stock-yards, and a number of animals showing the same combination of symptoms at the same time, a diagnosis is easily made in most cases. One symptom that may help to distinguish this disease from ordinary pneumonia is the conjunctivitis so frequently present. This starts as a simple conjunctivitis, with profuse lacrimation, and a pronounced puffiness of the conjunctivæ. The eye troubles may end here, or go on to a more severe inflammation of the conjunctivæ, extending into the cornea (keratitis) with or without ulceration. The discharge changes from a serous to a mucous, muco-purulent or purulent nature, sometimes closing the eyes by sealing the lids together. In a few cases only one eye will be involved in these further changes, suggesting that they may be due to traumatisms, or that they are caused by secondary infections.

The attention of the owner or herdsman is first drawn to the sick animals by their dull appearance and loss of appetite. Breathing may be a little quickened, and forced exercise causes some animals to cough. This is apparently painful to the animal, as it is suppressed, dry, and harsh. The pulse is accelerated, and in animals that have not become weak, the pulse is usually strong. In very sick animals the pulse is usually almost imperceptible. The temperature frequently goes over 107° F., but a majority of the

sick animals will show temperatures ranging from 103.5 to 105.0° F. Little satisfaction is gained from auscultation of the lungs. The pneumonic areas in the lungs are usually found in the apical and cardiac lobes, which are not accessible for auscultation. Increased breathing sounds are noted, as well as friction sounds in some cases. There may be a blood-tinged nasal discharge. Pleurodynia is in evidence, when animals are punched, or when pressure is applied, over the ribs.

The bowels may be constipated or loose. Some animals develop a very severe, fetid diarrhea. The appetite is irregular, and unless tempting food is offered, in the way of beets, carrots, or turnips, either cooked or raw, gruels or alfalfa, the animals will not eat at all. Rumination is suppressed entirely in a few cases. These animals will drool profusely, the saliva hanging in strings from the corners of the mouth, quite the same as in foot-and-mouth disease. Laymen have noted the fact that animals which reach the drooling stage rarely recover.

COURSE OF THE DISEASE.

Some animals are sick only a few days before death supervenes. Others will remain alive for a week or ten days before death ensues, due to exhaustion, coupled with toxemia. The course of the disease is practically unaffected by any form of medicinal treatment administered orally. Indeed, this fact early impresses itself upon the minds of owners of cattle so treated, the various remedies used by veterinarians faring about alike. Animals that become very sick, but eventually recover, are usually stunted for a while and rarely make profitable animals to feed. A small percentage of the animals attacked make quick, spontaneous recoveries. It is quite possible that these animals have no pulmonary complications or very limited involvement of any structures within the thoracic cavity. Such animals, however, are sometimes very sick for a few days and have temperatures running as high as any of the rest.

POST-MORTEM PATHOLOGY.

As is to be expected, the thoracic cavity is the seat of most of the important pathological changes. The amount of pleural fluid is increased and it is usually of a sero-gelatinous consistency.

The quantity varies from a few ounces to several quarts. The lungs show petechial hemorrhages and pneumonic areas. The latter are usually seen in the apical and cardiac lobes, less frequently in the diaphragmatic lobes. The lungs in these cases bear a marked resemblance to the lungs described in cases of contagious pleuro-pneumonia, in that there is a distinct "marbling" present. This is due to the edema present in the interlobular spaces of the lungs. Various stages of congestion, red and gray hepatization are present in the same lung. On section the larger air-passages are seen to contain a blood-tinged frothy material. The larger bronchioles show congestion of the mucous membrane. This sometimes extends up the bronchi into the trachea, and even to the larynx, and it is not unusual to find all of these structures filled with a frothy exudate. Edema of the throat has been noted in these cases, and usually there will be petechial hemorrhages on the laryngeal mucosa. Areas of pleurisy are frequently present, the pleura being very red and roughened by fibrin formation. The heart shows hemorrhages on the endocardium, sometimes petechial, but frequently extensive ecchymoses are noted. Less frequently hemorrhages are seen on the epicardium. The bronchial and mediastinal lymphnodes are enlarged, congested, and hemorrhagic. Lesions in other parts of the body are either absent or not characteristic, varying from case to case, with the secondary complications shown during the course of the disease.

TREATMENT.

As already stated, medicinal treatment is a failure. Kinsley[4] has reported very favorably on the results secured with bacterins prepared from the Bacterium bovisepticum. The same agent has been used successfully to immunize cattle against the infection, although in most cases where cattle are immunized in the yards before being shipped out, it is difficult to follow them up and get accurate reports of the result of vaccination. No news is usually taken as good news, and a falling off in the number of outbreaks reported is usually credited to the use of various immunizing products, little else being taken into consideration.

The variety of conditions under which these immunizing agents are used, and the present lack of any system for getting reliable data on the results obtained, render it difficult to place an

accurate value on these products for prophylactic purposes. When we come to a consideration of their use as curative agents, we likewise find all kinds of opinions expressed as to their merits. This is not strange when we consider, first of all, the variety of bacterins and vaccines at the disposal of the veterinarian, and in the second place the variety of conditions under which they are employed. Some veterinarians consider that bacterins and vaccines are absolutely contraindicated for treating sick animals. Among those who use them we find the greatest differences as to the matter of dosage and intervals between doses. In bacterial vaccine therapy, we should pay more regard to the number of organisms in the material injected than the size of the dose, as expressed in mils or cubic centimeters. One mil of a certain bacterin may contain as many organisms as four mils of another.

In the hope of finding an agent that might be of service for the treatment of animals already affected, the writer has prepared an anti-hemorrhagic septicemia serum. This is a hyperimmune serum obtained from horses highly immunized with large injections of virulent suspensions of Bacterium bovisepticum intravenously. The product protects small laboratory animals from fatal infection with virulent organisms injected simultaneously. About a dozen strains of the organism were used in the treatment of the horses, and their serum agglutinates some of these strains in rather high dilutions. Complement fixing substances are readily demonstrated in the serum of the horses after they have been on treatment for some time.

Several opportunities have been offered to try out the serum, in typical outbreaks of stock-yards pneumonia, during the past two years. Any opinions of its value as expressed in this paper are based purely on personal observations of the results obtained. In most cases these results have been highly satisfactory as far as the owners of the cattle were concerned. I do not hesitate to say that we appeared to save some very sick animals with large doses of the serum. In others, showing only early symptoms, these seemed to be aborted and the animals made uneventful recoveries following one injection of 50 to 60 mils of serum. Exposed animals not yet showing symptoms were given 30 to 40 mils of serum, and almost invariably these animals failed to come down with the disease, but in herds where we found it

possible to leave a number of untreated controls, we found that very few of these animals eventually took sick, if they were not already showing symptoms when first examined.

In one herd where the serum was administered to forty head, in various stages of the disease, all but two showed unmistakable signs of improvement within twenty-four hours, denoted by a fall in the temperature, improvement of the appetite and general appearance of the animals. Those cases which did not respond were the ones with severe gastro-intestinal disturbances. As much as 200 mils of serum have been given to a steer weighing 400 pounds, without saving the animal, while another of equal weight, and to all appearances just as sick, has pulled through with half that amount of serum. It is quite likely that these apparently inconsistent results will continue to be obtained with most antibacterial sera used as curative agents. It could hardly be otherwise with so many complicating factors.

There are many other points in connection with this disease that I would like to touch upon in this paper, but lack of time forbids me. Our investigations are being continued in the laboratory, and in the field as opportunities are presented. There is much still to be learned about this disease we have called stockyards pneumonia of cattle, as well as all of the other diseases which have been grouped under the name of hemorrhagic septicemia. In this paper I have tried to confine my remarks to but the one form of this disease, one that appears to be a distinct clinical entity. I have seen what appeared to be the same condition in a large band of sheep, numbering some 9,000.[5] On arrival at their destination these sheep were distributed to thirty different farms. The disease appeared in every lot, with considerable variation in the percentage of loss. In this outbreak, as well as all outbreaks of the disease among young cattle, the disease failed to spread to the native animals in a single instance. This may not be strange, when we keep in mind the fact that animals in the same shipment frequently fail to show any signs of the infection. Individual resistance and susceptibility undoubtedly play an important part in this disease.

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(Reprinted from the *Journal of the American Pharmaceutical Association*, Vol. 8, No. 11. November, 1919.)

DIGITALIS STANDARDIZATION: A CONSIDERATION OF CERTAIN METHODS OF BIOLOGICAL ASSAY.*

By L. W. Rowe.

The physiological standardization of the drugs comprising the digitalis series of heart tonics has received much consideration since Houghton[1] proposed the first method for the assay of Strophanthus preparations in 1898.

One of the more recent of the methods proposed for standardizing digitalis preparations, and one which constantly appears to be receiving consideration, is the cat method, which was suggested by Hatcher and Brody[2] in 1909. In this method the digitalis preparation, suitably diluted, is administered intravenously to an anesthetized cat. The degree of dilution and size of dose are such that, when slowly injected, the animal will be killed within 90 minutes. The result gives the M. L. D. based on the amount of the preparation necessary per kilogramme body weight of cat.

Several objections have been raised to the use of this method, the chief one being that the death of the cat is not always caused by the action of the digitalis on the heart, since respiration often ceases before the heart stops beating. Other objections are the irregularity in time of death of the test animal and the difficulty in obtaining a sufficient number of cats and in handling them.

The experiments reported in this paper were carried out, first, to determine whether any relationship exists between the results of assays by the cat and frog methods; second, to determine the accuracy of the cat method; and third, to suggest certain modifications of the method, in order to make it more practical for commercial assay work.

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*Read before the Scientific Section, A. Ph. A., New York Meeting, 1919.

A search of the literature reveals the fact that very little experimental work with the cat method has been reported except by Hatcher and his co-worker, Eggleston. Eckler's[3] work published in 1912 on this subject seems to prove that the heart of the cat stops beating before respiration ceases. The number of samples tested, however, is too small to prove whether the method is suitable for commercial testing. Rowntree and Macht[4] in their work by this method have recently suggested certain changes which seem to improve it. The rate of injection used by Rowntree and Macht is the most important of these changes and has been adopted in my experiments.

In a later publication, Hatcher[5] enumerates certain features which he claims as distinct advantages, namely, elimination of absorption, speed of obtaining results, ease of handling animals, and small comparative cost of assay. The method of administration certainly eliminates the question of absorption, which is an important factor in the U. S. P. frog method. The most important consideration, however, in selecting a method of assay is accuracy; this and cost involved in obtaining and handling cats is open to question.

While results can be obtained more rapidly than by the M. L. D. frog method it is doubtful if it is shorter than the official frog method. Most pharmacologists will agree that cats are not as easy to work with as other animals, but a suggestion will be made later which has seemed to facilitate the handling of the cats. As to the expense involved, the test animals cost 75 cents instead of 10 cents, as Hatcher states, and then cannot be obtained in sufficient numbers for ordinary experimental purposes. Raising cats is also very unprofitable, and our experience confirms that of Eckler[3] on this phase of the subject.

Eggleston's[6] work on the comparison of clinical results apparently established a ratio between the M. L. D. for cats and the dosage for man by carrying out clinical experiments in conjunction with his cat assays. It does not seem necessary, however, that results be transferable from the test animal to man in choosing a physiological method of assay, but other things being equal it is a point in favor of the cat method.

In my first experiments with the cat method, I attempted to kill the animal in as nearly 90 minutes as possible just as Eckler

did in his experiments. Later experiments showed, however, that much more uniform results could be obtained if the end point was reached in from 20 to 45 minutes, with 30 minutes as a good average. Also, in the first experiments the solutions were injected at a uniform rate from the beginning to the death of the animal. Injecting rapidly at first and then giving 1 mil every two minutes thereafter until the death of the animal is a better procedure.

In practically all of the experiments chloretone[7] was used as the anesthetic. It is easily given, is rapid in its action, and in every respect is very satisfactory. This is a very important point in the use of cats as the injection is given so easily and the action is so rapid and pronounced. For cats the solution used is only half as strong as that suggested for completely anesthetizing dogs, since a dose of 0.15 Gm. to 0.20 Gm. of chloretone per Kg. body weight is sufficient, when injected intraperitoneally, to produce a satisfactory anesthesia which does not affect the heart or depress the respiratory center, and requires no further administration of anesthetic after the first dose. The use of chloretone entirely eliminates the trouble experienced in administering anesthetics to cats, with the attendant danger of giving too much, though of course it does not overcome the other difficulty of working with these animals, namely, the insertion of a cannula into the small femoral vein. If chloretone is used as the anesthetic the only physical objection to the use of the cat as the test animal is the difficulty and even impossibility of obtaining them in sufficient numbers for assay purposes and the trouble of working with the small and delicate blood-vessels of this animal.

Because of the greater convenience in using dogs rather than cats, as well as the further advantages that dogs are more easily obtained and cheaper, I have made a series of tests of a number of samples, using the cat and the dog in the modified intravenous method, and comparing the activities thus obtained with the test of the sample by the minimum lethal dose frog method. The technique of the injection used in the later experiments, which is preferable to that of Hatcher, is that of Rowntree and Macht, in which approximately one-half of the calculated amount is injected at the rate of 3 mils per minute and the rest at the rate of 1 mil every two minutes. In the case of digitalis, ouabain was not used

to complete the reaction, as originally suggested by Hatcher. By using a larger dose, results equally as accurate can be obtained without the additional complication.

The dilutions chosen for injection should be such that the M. L. D. for cats is between 10 and 25 mils; for dogs between 20 and 50 mils, 18 mils being a good average total dose for cats and 25 to 30 mils being a good average total dose for dogs.

The following tables of data give the results of tests of 18 samples upon 61 cats and of 30 samples upon 132 dogs:

TABLE I.—EXPERIMENTS WITH CATS.

OUABAIN "A."

Cat No.	Sex.	Cond.	Weight.	Anes.	Dil. of sample.	Total dose.	Time to kill.	M. L. D. per Kg.
1	M	Good	2.12 Kg.	Deep	1 : 20000	3.9 mils	60 min.	0.092 mg.
2*	M	Good	2.72 Kg.	Deep	1 : 20000	6.4 mils	90 min.	0.1176 mg.
3	M	Good	2.47 Kg.	Deep	1 : 20000	4.7 mils	82 min.	0.095 mg.
5	F	Good	3.74 Kg.	Deep	1 : 20000	6.5 mils	75 min.	0.087 mg.
6*	M	Fair	1.73 Kg.	None given	1 : 20000	4.4 mils	50 min.	0.127 mg.

Average M. L. D. per Kg.=0.091 mg.

OUABAIN "B."

Cat No.	Sex.	Cond.	Weight.	Anes.	Dil. of sample.	Total dose.	Time to kill.	M. L. D. per Kg.
10	F	Fair	1.36 Kg.	Deep	1 : 50000	8.75 mils	46 min.	0.128 mg.
11	M	Good	2.1 Kg.	Light	1 : 50000	11.5 mils	33 min.	0.109 mg.
12	F	Good	2.82 Kg.	Deep	1 : 50000	18.5 mils	41 min.	0.131 mg.
13*	F	Poor (kitten)	0.86 Kg	Deep	1 : 50000	11.6 mils	38 min.	0.270 mg.
15*	M	Fair (kitten)	1.94 Kg.	Deep	1 : 50000	14.7 mils	36 min.	0.151 mg.
16*	F	Poor (kitten)	1.41 Kg.	Deep	1 : 50000	12.4 mils	48 min.	0.175 mg.

Average M. L. D. per Kg.=0.123 mg.

OUABAIN "C."

Cat No.	Sex.	Cond.	Weight.	Anes.	Dil. of sample.	Total dose.	Time to kill.	M. L. D. per Kg.
17	F	Good	2.74 Kg.	Deep	1 : 50000	21.9 mils	58 min.	0.160 mg.
18	M	Good	2.84 Kg.	Deep	1 : 50000	19.2 mils	65 min.	0.135 mg.
19	F	Poor	0.88 Kg.	Deep	1 : 100000	12.8 mils	40 min.	0.145 mg.

Average M. L .D. per Kg.=0.147 mg.

STROPHANTHIN (KOMBE) SAMPLE NO. 256490.

Cat No.	Sex.	Cond.	Weight.	Anes.	Dil. of sample.	Total dose.	Time to kill.	M. L. D. per Kg.
51	F	Good	2.40 Kg.	Fair	1 : 30000	15.0 mils	18 min.	0.228 mg.
52	F	Good	3.20 Kg.	Fair	1 : 30000	16.0 mils	16 min.	0.166 mg.
53	F	Good	2.70 Kg.	Fair	1 : 30000	15.0 mils	20 min.	0.185 mg.

Average M. L. D. per Kg.=0.186 mg.

TINCTURE OF STROPHANTHUS U. S. P. 1890.

Cat No.	Sex.	Cond.	Weight.	Anes.	Dil. of sample.	Total dose.	Time to kill.	M. L. D. per Kg.
20	F	Good	2.28 Kg.	Deep	1 : 100	13.3 mils	87 min.	0.059 mil
21	F	Good	2.60 Kg.	Deep	1 : 200	25.3 mils	47 min.	0.049 mil
22	F	Good	2.50 Kg.	Deep	1 : 100	14.1 mils	30 min.	0.056 mil
23	M	Fair	1.24 Kg.	Deep	1 : 100	6.44 mils	27 min.	0.052 mil
44*	M	Good	3.66 Kg.	Fair	1 : 100	26.0 mils	36 min.	0.071 mil

Average M. L. D. per Kg.=0.054 mil.

TABLE I.—EXPERIMENTS WITH CATS.

TINCTURE OF DIGITALIS FROM DRUG NO. 250139.

Cat No.	Sex.	Cond.	Weight.	Anes.	Dil. of sample.	Total dose.	Time to kill	M. L. D. per Kg.
24*	M	Good	2.00 Kg.	Deep	1 : 10	27.5 mils	30 min.	1.38 mils
25	M	Fair	1.40 Kg.	Deep	1 : 10	13.5 mils	21 min.	0.96 mils
26	F	Good	1.94 Kg.	Deep	1 : 10	18.2 mils	30 min.	0.94 mils
27	F	Good	2.16 Kg.	Deep	1 : 10	22.0 mils	60 min.	1.02 mils
	Average M. L. D. per Kg.=0.97 mil.							
				TINCTURE OF DIGITALIS "A."				
36	F	Good	2.92 Kg.	Deep	1 : 4	30.2 mils	30 min.	2.58 mils
				TINCTURE OF DIGITALIS "B."				
37*	M	Good	4.48 Kg.	Fair	1 : 4	27.0 mils	32 min.	1.50 mils
38	M	Good	3.00 Kg.	Light	1 : 5	21.0 mils	28 min.	1.40 mils
42	M	Small O. K.	1.60 Kg.	Light	1 : 5	11.0 mils	12 min.	1.38 mils
	Average M. L. D. per Kg.=1.40 mils.							
				TINCTURE OF DIGITALIS "C."				
39	F	Good	2.76 Kg.	Fair	1 : 5	15.0 mils	15 min.	1.09 mils
40	M	Good	2.75 Kg.	Fair	1 : 5	19.0 mils	25 min.	1.38 mils
41	M	Good	3.00 Kg.	Fair	1 : 5	19.0 mils	24 min.	1.27 mils
43	F	Good	3.35 Kg.	Light	1 : 5	23.0 mils	22 min.	1.37 mils
	Average M. L. D. per Kg.=1.28 mils.							
				DIGITALONE NO. 046798.				
34	F	Good	2.00 Kg.	Deep	1 : 10	17.9 mils	27 min.	0.90 mil
				FLUIDEXTRACT OF DIGITALIS, ℞ NO. 665561.				
48	F	Good	2.00 Kg.	Light	1 : 50	14.0 mils	14 min.	0.140 mil
49	M	Good	3.86 Kg.	Light	1 : 50	33.0 mils	37 min.	0.171 mil
50	F	Good	2.30 Kg.	Fair	1 : 50	15.0 mils	15 min.	0.130 mil
	Average M. L. D. per Kg.=0.147 mil.							
				FLUIDEXTRACT OF SQUILL, ℞ NO. 681685.				
45	M	Good	2.25 Kg.	Fair	1 : 50	13.0 mils	15 min.	0.115 mil
46	F	Good	2.35 Kg.	Fair	1 : 60	16.0 mils	20 min.	0.113 mil
47	F	Good	2.50 Kg.	Very light	1 : 60	16.0 mils	18 min.	0.107 mil
	Average M. L. D. per Kg.=0.112 mil.							

Asterisk after number of experimental animal means that result was not used in determining average.

TABLE II.—EXPERIMENTS WITH DOGS.

OUABAIN "A."

Dog No.	Sex.	Cond.	Weight.	Anes	Dil. of sample.	Total dose.	Time to kill.	M. L. D. per Kg.
1	F	Good*	7.05 Kg.	Deep	1 : 20000	16.5 mils	97 min.	0.117 mg.
2*	F	Good*	10.5 Kg.	Deep	1 : 40000	50.0 mils	Not fatal	
3	M	Good*	18.5 Kg.	Deep	1 : 20000	37.5 mils	85 min.	9.101 mg.
	Average M. L. D. per Kg.=0.109 mg.							
				OUABAIN "B."				
4	F	Good*	9.1 Kg.	Deep	1 : 25000	33.4 mils	57 min.	0.147 mg.
5	F	Good*	11.9 Kg.	Deep	1 : 20000	26.25 mils	60 min.	0.110 mg.
6*	M	Good*	12.05 Kg.	Deep	1 : 25000	48.2 mils	40 min.	0.160 mg.
7	F	Good	16.4 Kg.	Deep	1 : 20000	39.9 mils	38 min.	0.121 mg.
	Average M. L. D. per Kg.=0.126 mg.							

TABLE II.—EXPERIMENTS WITH DOGS (*Continued*).

Dog No.	Sex.	Cond.	Weight.	Anes.	Dil. of sample.	Total dose.	Time to kill.	M. L. D. per Kg.
				OUABAIN "C."				
11	M	Good*	9.0 Kg.	Deep	1 : 25000	28.6 mils	35 min.	0.127 mg.
12*	M	Fair*	7.0 Kg.	Deep	1 : 25000	28.8 mils	65 min.	0.165 mg.
13	M	Good*	9.5 Kg.	Deep	1 : 25000	33.0 mils	45 min.	0.139 mg.
14	F	Good*	17.2 Kg.	Deep	1 : 20000	50.0 mils	45 min.	0.145 mg.
15	F	Fair	6.0 Kg.	Deep	1 : 25000	30.4 mils	50 min.	0.136 mg.
16	M	Good*	9.0 Kg.	Deep	1 : 25000	33.5 mils	30 min.	0.149 mg.
Average M. L. D. per Kg.=0.139 mg.								
				STROPHANTHIN (KOMBE) No. 183774.				
21*	M	Good*	8.5 Kg.	Deep	1 : 10000	29.1 mils	45 min.	0.343 mg.
22	F	Good*	10.8 Kg.	Deep	1 : 10000	29.4 mils	20 min.	0.273 mg.
24*	M	Good*	13.4 Kg.	Deep	1 : 10000	50.0 mils	Not fatal	
25	F	Good*	15.0 Kg.	Deep	1 : 10000	36.4 mils	26 min.	0.242 mg.
30	F	Good*	8.2 Kg.	Deep	1 : 10000	20.5 mils	36 min.	0.250 mg.
Average M. L. D. per Kg.=0.255 mg.								
				STROPHANTHIN (KOMBE) No. 256490.				
98	M	Good	9.2 Kg.	Light	1 : 15000	35.0 mils	25 min.	0.253 mg.
99	F	Good*	12.8 Kg.	Fair	1 : 15000	45.0 mils	12 min.	0.234 mg.
100	M	Good	10.0 Kg.	Fair	1 : 15000	37.0 mils	28 min.	0.247 mg.
101*	M	Good*	14.4 Kg.	Deep	1 : 10000	41.0 mils	18 min.	0.284 mg.
Average M. L. D. per Kg.=0.245 mg.								
				STROPHANTHIN (KOMBE) No. 256491.				
86*	F	Good	7.56 Kg.	Fair	1 : 15000	32.0 mils	33 min.	0.282 mg.
89*	M	Good*	7.6 Kg.	Deep	1 : 15000	37.0 mils	35 min.	0.324 mg.
90*	F	Good	7.1 Kg.	Fair	1 : 15000	30.0 mils	20 min.	0.282 mg.
91	F	Good	8.4 Kg.	Deep	1 : 15000	30.0 mils	17 min.	0.238 mg.
92	M	Good	9.2 Kg.	Deep	1 : 15000	33.0 mils	24 min.	0.239 mg.
93	F	Good	10.5 Kg.	Light	1 : 15000	36.0 mils	20 min.	0.228 mg.
Average M. L. D. per Kg.=0.235 mg.								
				TINCTURE OF STROPHANTHUS U. S. P. 1890.				
17	M	Good	7.94 Kg.	Deep	1 : 50	27.0 mils	35 min.	0.068 mil
18*	F	Good*	19.5 Kg.	Deep	1 : 25	19.2 mils	28 min.	0.039 mil
19	F	Good*	10.45 Kg.	Deep	1 : 50	26.5 mils	38 min.	0.051 mil
20*	M	Good*	14.10 Kg.	Deep	1 : 50	26.8 mils	40 min.	0.038 mil
23*	M	Good*	16.7 Kg.	Deep	1 : 50	35.0 mils	60 min.	0.042 mil
74	M	Good*	9.8 Kg.	Fair	1 : 50	27.0 mils	32 min.	0.055 mil
75	M	Good*	13.1 Kg.	Deep	1 : 50	46.0 mils	53 min.	0.070 mil
76	F	Good*	10.7 Kg.	Deep	1 : 50	34.0 mils	36 min.	0.064 mil
77	F	Good*	8.3 Kg.	Deep	1 : 50	21.0 mils	37 min.	0.051 mil
78	M	Good*	17.5 Kg.	Light	1 : 50	47.0 mils	42 min.	0.054 mil
79	F	Good*	11.1 Kg.	Light	1 : 50	34.0 mils	31 min.	0.061 mil
83	M	Good*	19.0 Kg.	Deep	1 : 40	44.0 mils	45 min.	0.058 mil
84	M	Good*	15.2 Kg.	Deep	1 : 50	44.0 mils	38 min.	0.058 mil
106	M	Good*	10.4 Kg.	Deep	1 : 50	32.0 mils	31 min.	0.062 mil
Average M. L. D. per Kg.=0.059 mil.								
				TINCTURE OF STROPHANTHUS, U. S. P. 1910, ℞ 86068.				
63	M	Good*	20.7 Kg.	Deep	1 : 50	32.0 mils	31 min.	0.031 mil
64	M	Good	13.6 Kg.	Fair	1 : 50	21.0 mils	32 min.	0.031 mil
65	M	Good	15.0 Kg.	Fair	1 : 50	22.0 mils	20 min.	0.030 mil
66	M	Fair	14.2 Kg.	Light	1 : 50	23.0 mils	36 min.	0.032 mil
Average M. L. D. per Kg.=0.031 mil.								

TABLE II.—EXPERIMENTS WITH DOGS (*Continued*).

TINCTURE OF STROPHANTHUS, 1910, ℞ 683866.

Dog No.	Sex.	Cond.	Weight.	Anes.	Dil. of sample.	Total dose.	Time to kill.	M. L. D. per Kg.
118	M	Good	14.0 Kg.	Fair	1 : 50	15.0 mils	18 min.	0.021 mil
119	M	Good	10.2 Kg.	Fair	1 : 75	20.0 mils	25 min.	0.026 mil
120*	M	Fair *	6.75 Kg.	Deep	1 : 75	14.0 mils	14 min.	0.0276 mil
121	F	Good*	12.0 Kg.	Fair	1 : 100	25.0 mils	20 min.	0.021 mil
Average M. L. D. per Kg.=0.0227 mil.								
TINCTURE OF DIGITALIS FROM DRUG NO. 250139.								
26	M	Good*	9.75 Kg.	Deep	1 : 2	35.0 mils	86 min.	1.14 mils
TINCTURE OF DIGITALIS, ℞ 674678.								
31	F	Good*	9.75 Kg.	Deep	1 : 2	37.8 mils	42 min.	1.94 mils
32	M	Good*	7.07 Kg.	Light	1 : 25	34.5 mils	30 min.	1.95 mils
33*	F	Poor	4.75 Kg.	Light	1 : 3	31.0 mils	57 min.	2.18 mils
Average M. L. D. per Kg.=1.95 mils.								
TINCTURE OF DIGITALIS "A."								
35*	M	Good*	10.5 Kg.	Deep	1 : 2	42.0 mils	50 min.	2.0 mils
36	M	Good*	7.2 Kg.	Deep	1 : 3	59.0 mils	80 min.	2.73 mils
37	F	Good	7.75 Kg.	Fair	1 : 3	67.5 mils	85 min.	2.90 mils
38	M	Good	7.1 Kg.	Fair	1 : 2	44.0 mils	68 min.	3.09 mils
Average M. L. D. per Kg.=2.91 mils.								
TINCTURE OF DIGITALIS, ℞ 676593.								
49	F	Good*	8.8 Kg.	Fair	1 : 3	32.0 mils	50 min.	1.21 mils
50	M	Good*	10.7 Kg.	Deep	1 : 3	36.3 mils	50 min.	1.13 mils
Average M. L. D. per Kg.=1.17 mils.								
TINCTURE OF DIGITALIS, ℞ 676593 (ADJUSTED).								
52*	F	Good*	9.0 Kg.	Deep	1 : 3	45.0 mils	40 min.	1.66 mils
53	F	Good	6.1 Kg.	Light	1 : 3	33.0 mils	62 min.	1.80 mils
54*	F	Good*	10.0 Kg.	Deep	1 : 3	47.6 mils	27 min.	1.59 mils
56	F	Good	8.3 Kg.	Fair	1 : 3	49.0 mils	50 min.	1.97 mils
57	F	Good	9.65 Kg.	Fair	1 : 3	57.0 mils	58 min.	2.00 mils
Average M. L. D. per Kg.=1.92 mils.								
TINCTURE OF DIGITALIS "B."								
59	F	Good	6.9 Kg.	Fair	1 : 3	29.0 mils	33 min.	1.40 mils
85*	F	Very Poor	5.0 Kg.	Light	1 : 3	30.0 mils	42 min.	2.00 mils
87	F	Good	11.4 Kg.	Fair	1 : 3	50.0 mils	48 min.	1.46 mils
88*	F	Poor	5.8 Kg.	Fair	1 : 3	34.0 mils	36 min.	1.95 mils
No. 85 had distemper and No. 88 was mangy and emaciated.								
Average M. L. D. per Kg.=1.43 mils.								
TINCTURE OF DIGITALIS "C."								
60	M	Good*	12.1 Kg.	Deep	1 : 3	45.0 mils	40 min.	1.24 mils
61	M	Good	9.55 Kg.	Light	1 : 3	37.0 mils	37 min.	1.29 mils
62	F	Small*	7.9 Kg.	Deep	1 : 3	27.0 mils	20 min.	1.14 mils
Average M. L. D. per Kg.=1.22 mils.								
TINCTURE OF DIGITALIS, ℞ C136053.								
114*	M	Good*	11.0 Kg.	Deep	Undil.	14.0 mils	24 min.	1.27 mils
115	M	Good	13.6 Kg.	Deep	Undil.	21.0 mils	30 min.	1.54 mils
116	M	Good	7.75 Kg.	Fair	1 : 2	32.0 mils	46 min.	1.83 mils
117	M	Good*	14.6 Kg.	Deep	Undil.	22.0 mils	28 min.	1.50 mils
Average M. L. D. per Kg.=1.62 mils.								

TABLE II.—EXPERIMENTS WITH DOGS (*Continued*).

DIGITALONE, No. 046798.

Dog No.	Sex.	Cond.	Weight.	Anes.	Dil. of sample.	Total dose.	Time to kill.	M. L. D. per Kg.
27	M	Good*	6.8 Kg.	Deep	1 : 3	31.5 mils	105 min.	1.54 mils
28	M	Good*	8.8 Kg.	Fair	1 : 3	29.5 mils	70 min.	1.67 mils
29	M	Good*	9.4 Kg.	Deep	1 : 2	26.0 mils	30 min.	1.38 mils

Average M. L. D. per Kg.=1.53 mils.

DIGITALONE, No. 049780.

107	F	Good*	9.7 Kg.	Deep	Undil.	17.0 mils	20 min.	1.75 mils
108	M	Good*	13.35 Kg.	Fair	Undil.	23.0 mils	30 min.	1.73 mils
110	M	Good	8.8 Kg.	Light	1 : 2	32.0 mils	27 min.	1.81 mils

Average M. L. D. per Kg.=1.76 mils.

FLUIDEXTRACT OF DIGITALIS, ℞ 665561.

68	F	Good*	12.9 Kg.	Fair	1 : 20	47.0 mils	50 min.	0.186 mil
69	M	Good*	16.5 Kg.	Deep	1 : 10	29.0 mils	25 min.	0.176 mil
70*	M	Good	8.45 Kg.	Light	1 : 20	40.0 mils	52 min.	0.236 mil
71	M	Good	17.8 Kg.	Deep	1 : 10	31.0 mils	30 min.	0.174 mil
72	F	Small	6.25 Kg.	Light	1 : 20	25.0 mils	29 min.	0.200 mil

Average M. L. D. per Kg.=0.183 mil.

SOLID EXTRACT OF DIGITALIS, ℞ 661579.

80	M	Good*	20.0 Kg.	Fair	1 : 30	34.0 mils	48 min.	0.057 gm.
81	F	Good	10.0 Kg.	Deep	1 : 50	35.0 mils	50 min.	0.070 gm.
82	M	Good*	14.7 Kg.	Deep	1 : 30	26.0 mils	35 min.	0.059 gm.

Average M. L. D. per Kg.=0.062 Gm.

FLUIDEXTRACT OF SQUILL, ℞ 675384.

39*	F	Good*	13.0 Kg.	Deep	1 : 10	24.0 mils	65 min.	0.185 mil
40	M	Good	9.0 Kg.	Deep	1 : 15	21.2 mils	30 min.	0.156 mil
41*	F	Good*	11.2 Kg.	Deep	1 : 15	31.1 mils	48 min.	0.184 mil
42	F	Good	10.7 Kg.	Deep	1 : 15	25.8 mils	41 min.	0.160 mil
43*	F	Good*	13.5 Kg.	Deep	1 : 15	30.0 mils	34 min.	0.148 mil
44	M	Good	7.3 Kg.	Very light	1 : 15	17.0 mils	32 min.	0.156 mil
45	F	Good	9.0 Kg.	Very light	1 : 15	22.0 mils	60 min.	0.163 mil
48*	F	Good*	12.8 Kg.	Deep	1 : 15	36.3 mils	35 min.	0.190 mil

Average M. L. D. per Kg.=0.159 mil.

FLUIDEXTRACT OF SQUILL, ℞ 681685.

102	F	Good	15.9 Kg.	Deep	1 : 10	28.0 mils	20 min.	0.176 mil
103*	M	Poor	14.25 Kg.	Deep	1 : 10	20.0 mils	12 min.	0.140 mil
104	F	Fair	8.7 Kg.	Fair	1 : 20	28.0 mils	32 min.	0.161 mil
105	M	Good	10.0 Kg.	Deep	1 : 15	24.0 mils	19 min.	0.160 mil
109	F	Good	7.8 Kg.	Light	1 : 20	24.0 mils	27 min.	0.154 mil

Average M. L. D. per Kg.=0.163 mil.

FLUIDEXTRACT OF SQUILL, ℞ C134342.

55	M	Good*	14.5 Kg.	Deep	1 : 20	31.5 mils	27 min.	0.108 mil
58	M	Good*	12.6 Kg.	Deep	1 : 20	31.0 mils	36 min.	0.123 mil

Average M. L. D. per Kg.=0.115 mil.

Asterisk after number of experimental animal means that result was not used in determining average.

In Table II asterisk after condition of dog means that animal had been used prior to the test of the heart tonic.

Tables I and II give in as concise a form as possible all of the data which is necessary to a critical analysis of the results reported. An attempt was made to test a variety of preparations and yet to test several different samples of each type. The results are summarized in the following Tables III and IV:

TABLE III.—COMPARISON OF AVERAGE M. L. D. TO CATS AND DOGS.

Sample.	Cats. M. L. D. per Kg.	Dogs. M. L. D. per Kg.	Ratio.
Ouabain "A"	0.092 mg.	0.109 mg.	1 to 1.2
Ouabain "B"	0.123 mg.	0.126 mg.	1 to 1.02
Ouabain "C"	0.147 mg.	0.139 mg.	1 to 0.96
Ouabain "D"	0.134 mg.	0.151 mg.	1 to 1.12
Strophanthin			
No. 256490	0.186 mg.	0.245 mg.	1 to 1.31
No. 256491		0.235 mg.	
No. 183774		0.255 mg.	
Tr. Strophanthus			
U. S. P. 1890	0.054 mil	0.059 mil	1 to 1.09
℞ 86068		0.031 mil	
℞ 681309	0.0179 mil	0.0263 mil	1 to 1.47
℞ 683866	0.0179 mil	0.0227 mil	
Tr. Digitalis			
No. 250139	0.97 mil	1.14 mils	1 to 1.17
℞ 674678		1.95 mils	
"A"	2.58 mils	2.91 mils	1 to 1.12
℞ 676593		1.17 mils	
℞ 676593 (Adj.)		1.92 mils	
"B"	1.40 mils	1.43 mils	1 to 1.02
"C"	1.28 mils	1.22 mils	1 to 0.95
"D"	1.56 mils	1.82 mils	1 to 1.17
℞ C136053		1.62 mils	
Digitalone			
No. 046798	0.90 mil	1.53 mils	1 to 1.7
No. 049780		1.76 mils	
F. E. Digitalis			
℞ 665561	0.147 mil	0.183 mil	1 to 1.24
S. E. Digitalis			
℞ 661579		0.062 Gm.	
F. E. Squill			
℞ 673584		0.159 mil	
℞ 681685	0.112 mil	0.163 mil	1 to 1.45
℞ 134342		0.115 mil	
Tr. Digitalis Unknown Activity	0.98 mil	0.134 mil	1 to 1.37
Tr. Strophanthus Unknown activity	0.0286 mil	0.0326 mil	1 to 1.14
Ouabain Unknown activity	0.202 mg.	0.191 mg.	1 to 0.95

TABLE IV.

Sample.	Cat units.	Dog units.	M. L. D. frog method. Heart tonic units.
Ouabain "A"	10,989	9174	201,500 (101%)
Ouabain "B"	8,130	7936	185,200 (93%)
Ouabain "C"	6,802	7194	197,600 (98%)
Ouabain "D"	7,462	6622	177,800 (89%)
Strophanthin			
No. 256490	5,376	4081	150,000 (150%)
No. 256491		4255	171,000 (171%)
Tr. Stroph.			
U. S. P. 1890	18.5	17.0	650 (50%)
℞ 86068		32.2	815 (62.7%)
℞ 681309	55.86	41.1	1500 (115%)
℞ 683866		44.0	1978 (136%)
Tr. Digitalis			
No. 250139	1.03	0.88	10.0 (166%)
℞ 674678		0.51	5.0 (83%)
"A"	0.387	0.343	2.0 (33%)
℞ 676593		0.854	8.25 (137.5%)
℞ 676593 (Adj.)		0.52	6.67 (111%)
"B"	0.71	0.70	9.52 (158%)
"C"	0.78	0.82	6.06 (101%)
"D"	0.64	0.55	5.0 (83%)
℞ 136053	0.64	0.62	9.17 (153%)
Digitalone			
046798	1.1	0.65	5.9 (98%)
049780		0.57	6.6 (110%)
F. E. Digitalis			
℞ 665561	6.8	5.5	72.0 (110%)
S. E. Digitalis			
℞ 661579		16.1	140.0 (70%)
F. E. Squill			
℞ 675384		6.3	120.0 (150%)
℞ 681685		6.1	100.0 (125%)
℞ 134342		8.7	140.0 (175%)
Tr. Digitalis			
Unknown activity	1.02	0.746	8.0 (133%)
Tr. Stroph.			
Unknown activity	35.0	30.4	1000 (77%)
Ouabain			
Unknown activity	4950	5235	114,300 (57%)

Table III gives the average M. L. D. decided upon from the data obtained in the test of each sample upon either cats or dogs. Whenever the sample was tested upon both cats and dogs the ratio of the M. L. D. for the cat to that for the dog is placed in the fourth column. In this table it is plainly shown that there is no constant relation between the M. L. D. of a sample to cats and that of the same sample to dogs. In general, it can be stated, however, that the M. L. D. per Kg. body weight is slightly greater for the dog than for the cat.

Table IV gives the comparative results, in units, of the tests of samples upon the cat, dog and frog. The cat unit is defined by Hatcher as the amount of drug which is just sufficient to kill one kilogramme of cat when slowly and continuously injected into the vein. The number of units per gramme of the pure principles or per mil of tinctures or fluidextracts is, therefore, one divided by the average M. L. D. per Kg. as determined in the test. This exact procedure was also used in determining the number of dog units in each preparation. In the case of the M. L. D. frog test the Heart Tonic Unit is ten times the minimum lethal dose per gramme body weight of standard test frogs kept under proper test conditions. The number of heart tonic units per gram or per mil of a preparation is, therefore, one divided by 10 times the normal M. L. D. per gramme body weight of frog. The percentage which is placed in parentheses after the number of heart tonic units found for each sample is the strength of the sample in terms of the standard which has been adopted for that particular preparation.

This table (IV) shows that there is no definite relation between either the M. L. D. of a sample to the cat and M. L. D. to the frog or between the M. L. D. to the dog and that to the frog. In the case of the samples of ouabain, sample "C" was a close second in activity to sample "A" by the frog test, while by the cat and dog tests it was a poor third. The third sample of Tr. Strophanthus was three times as active as the first sample by the *cat* test and but 2.3 times as active by the frog test. The second sample of Tincture of Strophanthus was nearly twice as active as the first by the *dog* test while it was but 1¼ times as active by the frog test. Particularly in the tests of samples of Tincture of Digitalis are the inconsistencies of the M. L. D. to dogs plainly shown. One sample, ℞ 676593, was diluted on the basis of the original frog assay to 80 per cent of its original strength, and the assay of the diluted product by the M. L. D. frog test checked the dilution almost exactly, while the assay of these samples upon dogs showed the diluted product to be but 60 per cent as strong as the original. Several other instances of inconsistency between the results obtained upon dogs and frogs might be pointed out, but they can be readily seen upon close examination of the results.

In order to arrive at the relative accuracy of the three methods in as nearly an unprejudiced a manner as possible, and to check the results reported in the preceding tables, three samples were submitted for test. They were prepared by diluting or concentrating certain lots which had been tested by all three methods, but the degree of dilution or concentration was entirely unknown to the writer until after the tests were completed and results reported. Tables V and VI give the detailed reports of the tests as well as the comparison of the results obtained with the actual change which was made.

TABLE V.—ORIGINAL SAMPLES FROM WHICH UNKNOWNS WERE MADE.

TINCTURE OF STROPHANTHUS.

Test on Dogs.

No.	Sex.	Cond.	Weight.	Anes.	Dil of sample.	Total dose.	Time to kill.	M. L. D. per kg.
94*	F	Good	12.8 Kg.	Fair	1 : 100	30.0 mils	35 min.	0.0234 mil
95	M	Fair	7.2 Kg.	Fair	1 : 100	10.0 mils	26 min.	0.0277 mil
96	F	Good*	9.8 Kg.	Deep	1 : 100	26.0 mils	24 min.	0.0265 mil
97	M	Good	11.5 Kg.	Fair	1 : 100	29.0 mils	32 min.	0.0252 mil
112	F	Good*	11.6 Kg.	Deep	1 : 50	15.0 mils	14 min.	0.0259 mil

Average M. L. D. per Kg.=0.0263 mil.

Test on Cats.

No.	Sex.	Cond.	Weight.	Anes.	Dil of sample.	Total dose.	Time to kill.	M. L. D. per kg.
54	F	Good	2.7 Kg.	Light	1 : 250	12.0 mils	12 min.	0.0180 mil
55	F	Good	2.9 Kg.	Fair	1 : 250	13.0 mils	35 min.	0.0179 mil
56*	M	Good	3.3 Kg.	Fair	1 : 250	20.0 mils	38 min.	0.0242 mil
57	M	Good	3.75 Kg.	Fair	1 : 250	17.0 mils	25 min.	0.0180 mil
61	F	Good	2.45 Kg.	Fair	1 : 250	13.0 mils	21 min.	0.0177 mil

Average M. L. D. per Kg.=0.0179 mil.

TINCTURE OF DIGITALIS "D."

Test on Dogs.

No.	Sex.	Cond.	Weight.	Anes.	Dil of sample.	Total dose.	Time to kill.	M. L. D. per kg.
122*	F	Poor	8.5 Kg.	Fair	1 : 2	24 mils	30 min.	1.41 mils
123	M	Good	15.0 Kg.	Fair	1 : 2	50 mils	54 min.	1.66 mils
124	F	Good	6.1 Kg.	Fair	1 : 2	22 mils	37 min.	1.80 mils
125	M	Good	10.6 Kg.	Fair	1 : 2	41 mils	52 min.	1.93 mils
132	F	Good	9.6 Kg.	Fair	1 : 2	18 mils	35 min.	1.88 mils

Average M. L. D. per Kg.=1.82 mils.

Test on Cats.

No.	Sex.	Cond.	Weight.	Anes.	Dil of sample.	Total dose.	Time to kill.	M. L. D. per kg.
64	M	Fair	1.6 Kg.	Fair	1 : 6	15 mils	36 min.	1.56 mils
65	F	Fair	2.4 Kg.	Fair	1 : 6	20 mils	40 min.	1.39 mils
66	M	Fair	3.65 Kg.	Fair	1 : 6	38 mils	60 min.	1.73 mils

Average M. L. D. per Kg. 1.56 mils.

OUABAIN "D."

Test on Dogs.

No.	Sex.	Cond.	Weight.	Anes.	Dil of sample.	Total dose.	Time to kill.	M. L. D. per kg.
126	M	Good	9.9 Kg.	Fair	1 : 20000	30.0 mils	42 min.	0.151 mg.
127	F	Good	14.65 Kg.	Fair	1 : 20000	40.0 mils	25 min.	0.137 mg.
128	M	Good	17.1 Kg.	Fair	1 : 10000	28.0 mils	37 min.	0.164 mg.
129*	M	Good	12.4 Kg.	Light	1 : 10000	23.0 mils	34 min.	0.185 mg.

Average M. L. D. per Kg. 0.151 mg.

TABLE V.—ORIGINAL SAMPLES FROM WHICH UNKNOWNS WERE MADE (*Continued*).

OUABAIN "D"—*Continued*.

Test on Cats.

No.	Sex.	Cond.	Weight.	Anes.	Dil of sample.	Total dose.	Time to kill.	M. L. D. per kg.
67*	F	Kitten	1.3 Kg.	Fair	1 : 75000	17 mils	38 min.	0.174 mg.
68	F	Good	2.5 Kg.	Fair	1 : 50000	17 mils	26 min.	0.136 mg.
69*	F	Kitten	1.0 Kg.	Fair	1 : 50000	11 mils	30 min.	0.220 mg.
70	M	Good	3.05 Kg.	Fair	1 : 50000	20 mils	34 min.	0.131 mg.

Average M. L. D. per Kg.=0.134 mg.

SAMPLES (ACTIVITY UNKNOWN AT TIME OF TEST).

TINCTURE OF STROPHANTHUS FROM ℞ 681309.

Test on Dogs.

135	F	14.4 Kg.	Light	1 to 50	18 mils	24 min.	0.0250 mil
136	M	11.85 Kg.	Light	1 to 50	20 mils	38 min.	0.0337 mil
139*	M	8.2 Kg.	Fair	1 to 50	18 mils	31 min.	0.0440 mil
140*	M	10.6 Kg.	Fair	1 to 50	23 mils	35 min.	0.0434 mil
141	M	17.4 Kg.	Fair	1 to 50	29 mils	40 min.	0.0330 mil
142	M	10.6 Kg.	Fair	1 to 50	17 mils	20 min.	0.0320 mil
148	M	11.0 Kg.	Fair	1 to 75	26 mils	35 min.	0.0315 mil

Average M. L. D. per Kg.=0.0326 mil.

Test on Cats.

58	F	2.5 Kg.	Fair	1 to 250	18 mils	34 min.	0.0288 mil
59	M	3.75 Kg.	Fair	1 to 200	19 mils	24 min.	0.0253 mil
60	F	2.9 Kg.	Fair	1 to 250	23 mils	37 min.	0.0319 mil

Average M. L. D. per Kg.=0.0287 mil.

TINCTURE OF DIGITALIS FROM "D."

Test on Dogs.

133*	M	10.0 Kg.	Fair	1 to 2	22 mils	38 min.	1.1 mils
134	M	9.5 Kg.	Fair	1 to 2	23 mils	48 min.	1.31 mils
138	M	8.9 Kg.	Fair	1 to 2	23 mils	45 min.	1.29 mils
144	M	5.9 Kg.	Fair	1 to 3	25 mils	36 min.	1.41 mils

Average M. L. D. per Kg.=1.34 mils.

Test on Cats.

62	M	Good	3.2 Kg.	Fair	1 to 5	15 mils	20 min.	0.94 mil
63	M	Good	2.6 Kg.	Light	1 to 6	16 mils	30 min.	1.02 mils

Average M. L. D. per Kg.=0.98 mil.

OUABAIN FROM "D."

Test on Dogs.

143	F	11.4 Kg.	Light	1 to 15	34 mils	48 min.	0.198 mg.
146	M	9.0 Kg.	Fair	1 to 15	25 mils	33 min.	0.185 mg.
147	F	7.7 Kg.	Fair	1 to 15	22 mils	27 min.	0.190 mg.

Average M. L. D. per Kg.=0.191 mg.

Test on Cats.

71*	F	Kitten	1.2 Kg.	Fair	1 to 50000	15 mils	40 min.	0.250 mg.
72	M	Good	2.94 Kg.	Good	1 to 40000	25 mils	45 min.	0.213 mg.
73	F	Good	2.5 Kg.	Fair	1 to 40000	19 mils	34 min.	0.190 mg.

Average M. L. D. per Kg.=2.20 mg.

TABLE VI.—RESULTS OF TEST UNKNOWNS.

Sample.	% of Original. Dogs.	Cats.	Frogs.	Correct percent.
Tr. Stroph	74.5%	62.6%	66.7%	66.7%
Tr. Digitalis	135%	159%	160%	150%
Ouabain	79%	63%	64%	60%

From the results of the test of the unknowns reported in Table VI, it can be seen that the frog assay is the more reliable since in one case the report was exactly right, and in the other two slightly high (a matter of about 7 per cent). Two of the results on the dogs were considerably high, while the third was about as much too low, showing no consistency toward either high or low results. The results on the cats were much better than those on the dogs but not quite as good as those obtained by the frog method. Because of the fact that we have used the M. L. D. frog method for so many years to check up dilutions (based on original assays) of commercial lots and have with *very* few exceptions found it to be accurate, it seems that the results obtained from this small series of unknowns is entirely representative of the relative merits of the method.

Enough data was not obtained with cats to prove absolutely that they are as unsatisfactory as dogs, and in fact I scarcely believe that they are, but the data reported in Table IV indicates that there is no real consistency between the results obtained when using the cat and those obtained with the frog. Since the frog method vindicated itself so satisfactorily in the test of the unknowns it seems hardly possible that the cat method can be considered to possess the same degree of accuracy.

It, therefore, seems most logical to conclude from the results reported that no relationship exists between the M. L. D.'s of heart tonic preparations to cats, dogs and frogs, and that consequently, since the frog method has shown itself to be the most accurate by tests of samples of unknown activity, the M. L. D. frog method is the most accurate of the three. This being true, there should certainly be no hesitancy as to the choice of the method for use in quantitative assay work, even though it might possibly be proved that the cat or dog method is a little the less expensive and that results can be obtained in a somewhat shorter time than with the frog method.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 233, 1919.

(Reprinted from the *Journal of the American Pharmaceutical Association,* Vol. 8, No. 11, November, 1919.)

MAINTAINING FROGS FOR TEST PURPOSES.*

By L. W. Rowe.

The proper maintenance of a supply of normal frogs throughout the year, when fresh supplies are not available daily, requires special facilities to avoid excessive losses and to insure uniform results when standardizing preparations of heart tonics of the digitalis series by the frog methods.

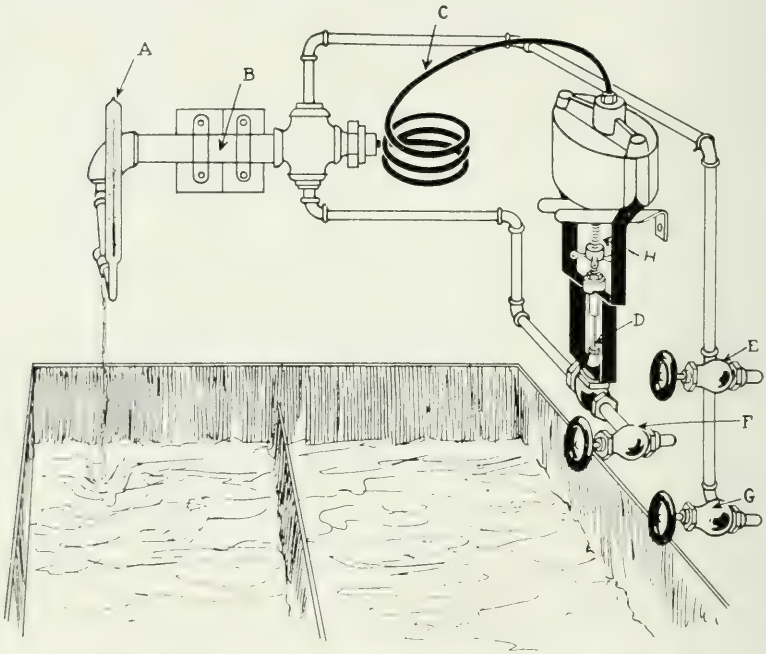
The chief source of trouble lies in the variation in temperature of the water in which the frogs are stored. In the summer the tap-water in the mains rises to 24° and 27° C., which is too warm, causing epidemics of disease to flourish among the frogs. In the winter the temperature of the tap-water goes as low as 4° C., and at this temperature the frogs are too sluggish. Furthermore, in the winter the sudden change of the frogs from the very cold water to that in which the tests are conducted is not uniformly responded to even if they are placed in the warmer water an hour or two before injections are made.

It was, therefore, thought desirable in our laboratory to control the temperature of the water entering the frog tank so that it would be the same during the entire year. The task becomes more complicated when it is realized that the tap-water must be artificially cooled in the summer and warmed in the winter.

The sketch represents the arrangement of the apparatus and the tank. The tank itself is made of copper and placed in a chamber 4 ft. x 4 ft. x 4 ft., five sides of which are sealed and insulated from room temperature by a thick layer of cork board. The sixth side is a tight-fitting refrigerator door with three spaced panes of glass. Since the door is opened only two or three times a day the air in the chamber is maintained at approximate-

*Read before Scientific Section, A. Ph. A., New York meeting, 1919.

ly the same temperature as the incoming water. From the sketch it can be seen that three pipe lines, for hot water (E), tap-water (G), and ice-cooled water (F), respectively, supply the tank. The refrigerator for cooling the water is located below the sealed chamber. The thermometer (A) shows the temperature of the water entering the tank.



Apparatus and Tank for Maintaining Frogs for Test Purposes.

The apparatus which controls the temperature of the water which enters the tank is known as the Sylphon Tank Regulator and is made by the American Radiator Co. A bulb containing a liquid with a high coefficient of expansion is situated in a metal jacket (B) through which the final mixture of warm and cold water must pass. This metal jacket is connected by pressure tubing (C) with a needle valve (D) in the ice-cooled line. The expansion or contraction of the liquid in the bulb controls the amount of cold water which is admitted by opening or closing the valve. A spring (H) connected with the needle valve can be

tightened or loosened so that any desired temperature will be necessary to open or close the needle valve.

In summer the hot water is not used at all, but enough ice-cooled water is let in by the needle valve to cool a small stream of tap-water to the desired temperature. In winter the temperature of the tap-water is raised to a point a little above that required, by opening the hot water valve slightly; then the needle valve lets in enough water from the cold water line (which in the winter is equivalent to a tap-water line since no ice is placed in the refrigerator) to cool the stream to the desired temperature.

To comply with specifications in the U. S. P., frogs must be kept at 15° C. until wanted for immediate use.

It has been found in this laboratory that this temperature (15° C.) is well suited to the proper storage of normal frogs, and with the apparatus described above and the arrangement of it in the sealed chamber we are able to maintain this temperature to within about 1° C. throughout the entire year, if necessary. While very sensitive to improper handling it is equally susceptible to proper adjustment, and has been found satisfactory for maintaining an even temperature at all seasons.

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 234, 1919.

(Reprinted from *The Journal of the Michigan State Medical Society*, November, 1919.)

EPIDEMIC CEREBROSPINAL MENINGITIS AT CAMP JACKSON, S. C.

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The first case of cerebrospinal meningitis at Camp Jackson, S. C., was diagnosed Nov. 15, 1917. The disease assumed the character of an epidemic during December, and January and February of the year following.

PREDISPOSING FACTORS.

The rapid spread of this disease through the camp was due to the following predisposing circumstances:

1. The unusual cold weather with sudden, marked temperature changes during the later fall and winter of 1917-18, to which the majority of men were not accustomed, and which caused a widespread nasopharyngeal catarrh, bronchitis, mumps, and measles.

Tables 1, 2, 3, and 4 give the weather conditions during the months of November, December, 1917, and January and February of the year following.

2. The general condition of the camp, which was not completed until the end of the winter. The facilities for heating the buildings were especially retarded. To keep warm, the men would stay indoors, crowding the barracks, thus coming in closer contact with those suffering from nasopharyngeal inflammations.

The majority of the barracks rooms were heated by one large furnace, the size of a hot-air furnace used for heating houses. Usually two such furnaces were installed in the center of the two rooms on the ground floor. As there were no pipes for the distribution of the heat throughout the building, during spells of cold weather the men would congregate around these furnaces, thus offering every advantage for the spread of respiratory infections.

3. The base hospital was not completed, and while every attempt was made to accommodate all, the problem became more involved, because white and negro patients had to be kept separate.

4. The laboratory facilities were meager. The function of the base hospital laboratory as a public health laboratory for the entire camp had not been taken into consideration in the plans of the hospital. This was corrected during the later part of January both as regards space and equipment.

5. The transfer of troops from one cantonment to another. By this means new foci of infection were introduced, and the problem of coping with the condition present complicated. The following cases are illustrative of this:

(a). Nov. 18, 1917. D. S., Pvt., Co. 21, 156 D. B. from Camp Pike, Little Rock, Ark., taken from train directly to hospital, diag. C. S. M.

(b). F. M., Pvt., Co. 23, 156 D. B. from Camp Pike, Little Rock, Ark., arrived at Camp Jackson Nov. 17, 1917; admitted to Base Hosp., C. S. M. No. 21, 1917.

(c). P. B., Pvt., Co. 15, 156 D. B. from Camp Gordon, Atlanta, arrived at Camp Jackson, S. C., Nov. 5, 1917. Admitted to Base Hosp. as C. S. M. Suspect, Nov. 8, 1917; diagnosis pos. C. S. M., Nov. 10, 1917.

(d). W. F. F., Pvt., Co. 21, 156 D. B. from Camp Pike, Little Rock, Ark, arrived at Camp Jackson, S. C., Dec. 1, 1917; diagnosis C. S. M.

BACTERIOLOGY.

While it is highly probable that local inflammation of the nasopharynx may be caused by the meningococcus before its invasion becomes general, its exact etiologic significance in relation to the inflammation of the respiratory passages is still doubtful, for its presence on the inflamed nasopharyngeal membrane may be accidental. The absence of marked inflammation of the nasopharyngeal membranes in some cases of meningitis throughout the entire course of the disease further supports this view. The assumption that the meningococcus reaches the meninges from the nasopharynx by way of the cribriform plate of the ethmoid is also questionable in view of the findings of Westenhoffer, Von Lingelsheim and Meyer, which indicate that if direct extension does occur, its course is through the sphenoid bones, the sinuses of which were found inflamed in 34 per cent of cases examined.

That the meningococcus produces a local inflammation before invading the general system may be assumed until further data are available. From the nasopharynx the organism invades the blood current, either directly, causing a meningococcus bacteremia with secondary localization in the meninges, or by a direct infection through the lymphatics, as pointed out by Westenhoffer, who observed the constant enlargement of the cervical lymph glands.

TABLE I.

Weather conditions during the month of November, 1917, as given by:
U. S. Department of Agriculture

Weather Bureau. C. F. Marvin, Chief.

Date	Highest	Temperature Lowest	Mean	Precipitation in inches and hundredths	Character of day
1	58	38	48	.00	Clear
2	55	38	46	.00	Clear
3	57	32	44	.00	Clear
4	60	39	50	.00	Clear
5	60	36	48	.00	Clear
6	67	34	50	.00	Clear
7	75	43	59	.00	Clear
8	67	44	56	.00	Clear
9	71	40	56	.00	Clear
10	71	42	56	.00	Clear
11	73	43	58	.00	Clear
12	71	46	58	T.	Pt. Cloudy
13	63	51	57	.52	Cloudy
14	55	49	52	.02	Cloudy
15	64	45	54	.00	Clear
16	62	44	53	.00	Pt. Cloudy
17	66	36	51	.00	Clear
18	71	45	58	.00	Clear
19	73	47	60	.00	Clear
20	54	46	50	.08	Cloudy
21	68	49	58	.00	Clear
22	67	49	58	.00	Clear
23	54	38	46	.00	Pt. Cloudy
24	45	32	38	.00	Pt. Cloudy
25	46	26	36	.00	Clear
26	50	26	38	.00	Clear
27	55	34	44	.00	Clear
28	62	39	50	.00	Cloudy
29	65	52	58	.21	Cloudy
30	55	48	52	.09	·Cloudy

WEATHER.

Number of clear days, 20; partly cloudy, 4; cloudy, 6; on which 0.01 inch, or more, of precipitation occurred, 5.

Mean relative humidity; 8 a. m., 81.7 per cent.; 8 p. m., 36.6 per cent.; monthly, 59.2 per cent.

Hail 13
Sleet 0
Fog, slight 15, 30
Fog, dense 29, 30
Thunderstorms 13
Frost, light 2
Frost, heavy 1
Frost, killing 3

TABLE II.

Weather conditions during the month of December, 1917, as given by:
U. S. Department of Agriculture
Weather Bureau. C. F. Marvin, Chief.

Date	Highest	Temperature Lowest	Mean	Precipitation in inches and hundredths	Character of day
1	65	45	55	.00	Clear
2	70	43	56	.00	Clear
3	72	51	62	.00	Pt. Cloudy
4	71	56	64	.17	Pt. Cloudy
5	59	44	52	.01	Cloudy
6	45	38	42	T.	Cloudy
7	52	31	42	.00	Clear
8	61	30	46	.27	Pt. Cloudy
9	31	20	26	.00	Clear
10	32	22	27	.00	Clear
11	35	19	27	.03	Pt. Cloudy
12	31	25	28	.01	Cloudy
13	28	24	26	.02	Cloudy
14	38	20	29	.00	Pt. Cloudy
15	34	22	28	.00	Clear
16	30	25	28	.02	Cloudy
17	38	26	32	.00	Clear
18	34	31	32	.00	Cloudy
19	44	33	38	.00	Cloudy
20	49	35	42	.00	Pt. Cloudy
21	61	30	46	.00	Clear
22	52	34	43	.00	Pt. Cloudy
23	48	30	39	.00	Clear
24	61	29	45	.00	Clear
25	64	40	52	.23	Cloudy
26	53	31	42	.36	Cloudy
27	34	25	30	.00	Cloudy
28	50	30	40	.00	Clear
29	39	18	28	.04	Cloudy
30	22	8	15	.00	Clear
31	21	12	16	.08	Cloudy

WEATHER.

Number of clear days, 12; partly cloudy, 7; cloudy, 12; on which 0.01 inch, or more, of precipitation occurred, 11.

Mean relative humidity: 8 a. m., 80.7 per cent.; 8 p. m., 64.5 per cent.; monthly, 72.6 per cent.

Sleet 11, 12, 13
Fog, slight 14
Fog, dense 1
Thunderstorms 0
Frost, light .... —
Frost, heavy .... —
Frost, killing .... —

TABLE III.

Weather conditions during the month of January, 1918, as given by:
U. S. Department of Agriculture
Weather Bureau. C. F. Marvin, Chief.

Date	Highest	Temperature Lowest	Mean	Precipitation in inches and hundredths	Character of day
1	29	6	18	.00	Clear
2	34	26	30	.07	Cloudy
3	30	15	22	.00	Clear
4	38	13	26	.00	Pt. Cloudy
5	52	31	42	.00	Cloudy
6	54	42	48	.19	Cloudy
7	53	31	42	.00	Clear
8	42	26	34	.00	Clear
9	44	30	37	T.	Cloudy
10	50	25	38	.00	Clear
11	64	30	47	.41	Cloudy
12	51	15	33	.00	Clear
13	31	10	20	.00	Clear
14	52	22	37	.25	Pt. Cloudy
15	57	34	46	.23	Clear
16	51	30	40	.00	Cloudy
17	51	31	41	.00	Clear
18	43	28	36	.00	Clear
19	41	24	32	.00	Clear
20	40	31	36	.08	Cloudy
21	35	26	30	.37	Cloudy
22	39	27	33	T.	Cloudy
23	44	25	34	.00	Clear
24	49	27	38	.00	Clear
25	63	39	51	.00	Clear
26	65	41	53	.00	Pt. Cloudy
27	71	57	64	.00	Cloudy
28	63	39	51	.34	Cloudy
29	54	39	46	.03	Cloudy
30	47	35	41	.17	Cloudy
31	42	33	38	.02	Cloudy

WEATHER.

Number of clear days, 14; partly cloudy, 3; cloudy, 14; on which 0.01 inch, or more, of precipitation occurred, 11.

Mean relative humidity: 8 a. m., 78.2 per cent.; 8 p. m., 66.2 per cent.; monthly 72.2 per cent.

Hail	0
Sleet	20
Fog, slight	28, 29
Fog, dense	0
Thunderstorms	11
Frost, light	—
Frost, heavy	—
Frost, killing	—

TABLE IV.

Weather conditions during the month of February, 1918, as given by:
U. S. Department of Agriculture

Weather Bureau. C. F. Marvin, Chief.

Date	Highest	Temperature Lowest	Mean	Precipitation in inches and hundredths	Character of day
1	40	35	38	T.	Cloudy
2	39	36	38	.56	Cloudy
3	57	39	48	.26	Cloudy
4	45	31	38	.00	Clear
5	41	25	33	.00	Pt. Cloudy
6	58	25	42	.00	Clear
7	67	48	58	.00	Pt. Cloudy
8	74	45	60	.00	Clear
9	73	52	62	.03	Pt. Cloudy
10	69	55	62	.00	Clear
11	74	43	58	.00	Clear
12	73	50	62	.22	Cloudy
13	74	60	67	.00	Pt. Cloudy
14	76	53	64	T.	Pt. Cloudy
15	77	62	70	.00	Pt. Cloudy
16	63	43	53	.21	Cloudy
17	59	47	53	.02	Cloudy
18	49	38	44	.06	Cloudy
19	72	44	58	.01	Cloudy
20	67	52	60	.05	Cloudy
21	52	38	45	.00	Pt. Cloudy
22	55	36	46	T.	Cloudy
23	58	36	47	.00	Clear
24	70	43	56	.00	Pt. Cloudy
25	74	58	66	.00	Pt. Cloudy
26	70	51	60	.00	Clear
27	72	39	56	.00	Clear
28	81	51	66	.00	Clear

WEATHER.

Number of clear days, 9; partly cloudy, 9; cloudy, 10; on which 0.01 inch, or more, of precipitation occurred, 9.

Mean relative humidity: 8 a. m., 79.4 per cent.; 8 p. m., 57.6 per cent.; monthly, 68.5 per cent.

Sleet	0
Fog, light	1, 8, 12, 27, 28
Fog, dense	0
Thunderstorms	16
Frost, light	—
Frost, heavy	—
Frost, killing	—

That this disease may at times be a generalized infection has been shown by Solomon, Moller, Bettencourt, Franc, and Elsner, who have in isolated instances succeeded in culturing the meningococcus from the blood. Elsner succeeded in isolating the organism in 25 per cent of cases. Routine blood cultures on twenty-three patients, meningitis suspects, taken on admission to the base hospital, Camp Jackson, S. C., gave positive cultures in 36.6 per cent.[1] As pointed out these cultures were obtained in many instances before the diagnosis of cerebrospinal meningitis was established and before meningeal symptoms were noticeable.

All the strains of meningococci isolated either from the nasopharynx, spinal fluid, blood and secondary foci were typed. In all instances we were dealing with a meningococcus of the regular type.

While generally cerebrospinal meningitis is diagnosed from the symptoms resulting from meningeal irritation, there is observable in many cases a premeningeal stage of the disease which varies in time from several days to a few hours, and is characterized by general septic symptoms, as chills, fever, malaise, lack of appetite, indefinite pains in the joints and muscles. The headache observed in this stage is probably due to increased amounts of cerebrospinal fluid, which on lumbar puncture is found clear, containing no organisms or only a few free meningococci which may be explained by the general sepsis. The cytology of the fluid may be negative or may show a slight increase in lymphocytes. The globulin test may be negative or faintly positive. Fehling's solution is reduced. The essential impression gained from the examination of the fluid during this stage of the disease is not that of meningitis, but rather that due to a general toxemia.

STAGES OF INVOLVEMENT.

The stage of meningeal involvement has been variously subdivided according to severity of the disease, its symptom-complex and probable prognosis.

This general bacteremia leads to widely scattered foci of infection in the body, of which that of the meninges is the most

1. Cultivation of the meningococcus intracellularis (Weichselbaum) from the blood. Baeslack, F. W.; Bunce, A. H.; Brunelle, G. C.; Fleming, J. S.; Klugh, G. F.; McLean, E. H.; Solomon, A. V. Jour. Am. Med. Asso., 1918, 70, 684.

commonly observed. The following cases are illustrative of the metastatic involvement of other regions of the body than the meninges:

1. Pvt. J. M. F., aged 25, admitted Jan. 5, 1918, was in poor condition; there were marked tremor, and cyanosis of the lips and finger-tips. The provisional diagnosis was bronchitis following measles, Jan. 11; broncho-pneumonia developed. The patient died Jan. 15. Cultures of pus found at necropsy, in the sphenoid sinus, gave a gram-negative diplococcus, which was identified as a meningococcus of the regular type.

2. S. R., Pvt., aged 23, admitted Nov. 15, 1918, complained of cold and pain in chest, and pain in the eyes and back. The provisional diagnosis was measles. Nov. 24, bronchitis developed in the left side. Nov. 28, there was pleurisy on the left side. Next day the diagnosis of broncho-pneumonia was made. Jan. 15, 1918, an intercostal incision for left emphysema was made and a large amount of slightly cloudy serous fluid removed. A meningococcus of the regular type was isolated from it.

3. Chas., Pvt., 156 D. B., Co. 19, was admitted as meningitis suspect. One lumbar puncture made, examination of spinal fluid negative. Patient developed mumps, and was transferred from wards for meningitis cases to that for mumps. To determine whether patient had had meningococcic infection, sample of blood was obtained. The serum from this blood agglutinated a strain of meningococcus of the regular type up to 1:320 dilution.

4. Miss M., nurse, reported on sick list, diagnosis articular rheumatism, which did not yield to usual treatment. On suggestion of the Chief of the Laboratory, a blood culture was taken, disclosing a gram-negative diplococcus, which was identified as a meningococcus of the regular type. Patient was put on antimeningococcic serum treatment, receiving it intravenously, and recovered promptly.

These instances clearly show that the meningococcus is distributed by the blood stream, giving rise to lesions wherever conditions are favorable, without necessarily involving the meninges. To the same mechanism of distribution may be credited the complications and sequelæ incident to this disease, as involvement of the joints, pleurisy, pericarditis, ophthalmitis, etc.

COMPLICATIONS.

The thirty-one autopsies performed during the height of the epidemic gave further evidence of the generalized nature of the meningococcus infection. The following gross lesions were recorded in the number of cases indicated:

Petechiæ	11
Purpura	10
Pleurisy	8
Pneumonia, Lobar	2
Pneumonia, Broncho	8
Congestion of the lung	7
Hypertrophy of heart	1
Dilatation of heart	1
Endocarditis, vegetative	1
Pericarditis, fibropurulent	4
Pericarditis, seropurulent	4
Spleen, enlarged	22
Kidneys, cloudy swelling	10
Kidneys, congestion	10
Purulent exudate in vertex	7
Purulent exudate in base	3
Purulent exudate in vertex and base	16
Purulent exudate in parietal region	5

While diffuse purpuric spots are absent on the serous surfaces, petechiæ were frequently observed on these membranes of the pericardium and peritoneum. It may be assumed that cerebrospinal meningitis is in more instances than has been supposed a bacteremia coexistent with or followed by the formation of metastatic foci due to the distribution of the meningococcus by the blood stream.

This assumption is based on the following considerations:

1. The demonstration of the meningococcus in blood cultures in a larger percentage of cases than heretofore.
2. The presence of a distinct premeningeal stage of the disease.
3. The occurrence of lesions due to the meningococcus in parts other than the meninges.
4. The autopsy findings, with special reference to the occurrence of petechiæ and purpuric spots.

Based on these observations the intravenous serum therapy in large doses was recommended by us to Major W. W. Herrick, the Chief of the Medical Service.[2] The agglutinating titer of most antimeningococcic sera lies between 1:800 to 1:1000. It will be

2. For a discussion of the intravenous serum treatment of cerebrospinal meningitis and the results obtained, the reader is referred to the article by W. W. Herrick, Major, M. R. C. The intravenous serum treatment of Epidemic Cerebrospinal Meningitis. Arch. Int. Med., Vol. XXI, 1918, p. 541.

seen that fairly large quantities of serum must be administered intravenously to have the therapeutic agent in sufficient concentration to act upon the meningococci present in the blood stream.

STATISTICS.

Two hundred and fourteen meningitis patients from 97 organizations were admitted to the base hospital. Out of this number 65 died, 88 were returned to duty shortly after dismissal from the hospital, 57 requiring additional time for complete recuperation; 4 were discharged; 2 cases recurred.

Twenty-eight of the patients were city residents before entry into the National Army, 159 were rural, the residence of 27 could not be determined.

PROPHYLAXIS.

The prophylactic measures instituted consisted of:

(a) The isolation of those complaining of symptoms observed in the premeningeal stage of the disease.

(b) The placing of the organization in quarantine.

(c) Preventing the men from congregating in the barracks.

(d) Alternating the position of the cots.

(e) Culturing the quarantined personnel 3 or more times at 5 day intervals, or until no more carriers could be found.

(f) Culturing all patients on admission to the hospital and segregating those found to be carriers in the hospital.

(g) The culturing of all applying for leave of absence from the camp.

(h) The isolation and treatment of the carriers in the carrier camp.

The vigilance exercised by the regimental surgeons in isolating those complaining of headache, malaise, chill, indefinite pains in muscles and joints combined with inflammation of the respiratory tract, made possible the prompt treatment of meningitis patients, while still in the early stages of the disease.

The extent of the quarantine was determined by the number of men the suspected case had come into contact with, just previous to his illness. Depending on this a single squad room, or barrack or the entire organization, would be placed under the quarantine.

The infecting organism of cerebrospinal meningitis gains access

to the respiratory passages by the inhalation of the fine spray droplets produced in the act of sneezing and coughing. The large number of men suffering from coryza, pharyngitis and bronchitis would aid materially in spreading the meningococcus infection in crowded rooms, provided contacts and carriers were among them. Hence the men were kept out of doors as much as possible, and the seating in the mess rooms was so arranged that the men occupied alternate seats. Crowding the barracks wherever it occurred was relieved, and the cots so arranged that no two men slept side by side with the heads in the same direction, but alternating.

BACTERIAL DIAGNOSIS.

As soon as diagnosis was established by the laboratory, name, rank and organization were reported to the office of the Division Surgeon. All contacts were quarantined and arrangements made for the taking of cultures. A list of those quarantined, made in triplicate, was furnished the culturing team, consisting of three physicians detailed to this duty by the Division Surgeon.

This team would obtain the necessary number of blood-agar plates, pack them into a fireless cooker and visit the organization for culturing. The plates were numbered and the corresponding number entered next to the name on the rosters prepared. The cultures and rosters were delivered at the laboratory, and on conclusion of the examination those found to be carriers were entered as positive on the lists, one of which was forwarded to the office of the Division Surgeon, one to the organization, the third being retained at the office of the laboratory for record.

The cultures were incubated for 18-24 hours, examined, and transplants of suspicious colonies made by three members of the laboratory staff. Agglutination tests were made on all suspicious cultures. The media used consisted of laked blood, 1 per cent glucose-agar.

At first the West tube was used for taking cultures; this, however, was found impracticable owing to the large number of cultures which had to be taken daily and to the time required for cleaning, refitting, and sterilizing these tubes. Our next method of taking cultures was by means of sterilized wooden applicators. A small pledget of cotton wound on the end of these applicators permitted the culturing through the nose, thus reaching the

posterior nasopharyngeal wall. The last method employed by us consisted of applicators made from No. 18 stove wire. The wire is cut up into 8-10 inch lengths, a loop is bent on one end, and the other end hammered flat so that cotton wound around this end will hold. Five of the applicators are placed into a heavy walled test tube, plugged and sterilized. When ready for culturing, the wire is bent on the edge of the test tube to an angle of approximately 60 degrees, one and one-half inches from the end holding the cotton. By burning off the cotton and straightening out the wire it may again be prepared for use. The reduced expense and saving of time, as well as the ease with which cultures can be taken, recommend its use.

The culturing was done in a convenient room which had been washed and cleaned previously. A table and chair for the clerk, a pail and a chair so placed that the person to be cultured faces the light, are all the furniture necessary. One man at a time is allowed to come into the room to prevent crowding. The tongue is depressed with a wooden tongue depressor and the culture taken by introducing the wire applicator well up the posterior portion of the nasopharynx, first on one side of the uvula and then on the other.

The applicator is withdrawn without touching the oral cavity and the culture plate inoculated, and the inoculated material evenly spread with a platinum wire.

The fireless cooker keeps the plates and cultures at constant temperature until placed in the incubator.

Owing to shortage in glassware we were compelled at first to put three cultures on each plate. This number was later reduced to two, as more petri dishes were available.

The agglutinations were carried out with one or two polyvalent sera, diluted 1:200 and normal horse serum diluted 1:50. All strains of meningococci isolated either from the spinal fluid, blood, or nasopharynx as well as those obtained from exudates of patients were typed. All stains thus examined were of the "regular" type. The type sera used were furnished by the Army medical school.

ISOLATION.

Upon notification from the laboratory, the men found to be carriers were isolated in the carrier camp, established by orders

of the Division Surgeon. Here a card index was kept of the date of entry, number of cultures taken and the results of the culture. Three negative cultures obtained in succession, at from 3 to 5 day intervals, were required before discharge from the carrier camp.

TREATMENT.

The treatment of the carriers consisted in periodical spraying of the upper air passages with dichloramine-T. Treatment was discontinued at least 18 hours before culturing.

During the period from December 18, 1917, until March 7, 1918, 520 carriers were isolated and treated in the carrier camp. Of this number six developed cerebrospinal meningitis.

The number of cultures taken from organizations during the months indicated are as follows:

Dec., 1917	4873	Carriers isolated 176—3.6 %
Jan., 1918	11825	Carriers isolated 293—2.47%
Feb., 1918	2480	Carriers isolated 51—2.06%

In all 97 organizations were involved in this epidemic. Of this number 59 were cultured, 38 were not cultured, since in the latter one case only of cerebrospinal meningitis appeared. In addition 22 organizations were cultured for the purpose of removing the carriers before cases could develop.

Aside from the cultures taken in organizations and the carrier camp, it was thought advisable to culture all patients at the time of admission to the hospital. This measure was instituted to prevent the spread of meningitis to other than meningitis wards of the hospital by isolating patients who were carriers of the meningococcus. As soon as the patient was admitted through the receiving ward, he was, if able to walk, brought to the laboratory where the culture was taken; if unable to walk, the culture was taken at the receiving ward. From 75 to 125 cultures were thus taken daily at the laboratory. This number includes also those who desired leave of absence. A statement that bearer had been cultured and found negative was required before permission to leave the camp was granted.

The spread of cerebrospinal meningitis is mainly due to the carriers, who, under certain conditions, such as inflammation of the upper respiratory tract, harbor the meningoccus in a more or less virulent form. The coexistence of measles, coryza and bronchitis

with cerebrospinal meningitis is more than accidental, playing an important role in the infectivity of the meningococcus, and the establishing of new foci of the disease. The removal of the carriers is usually followed by a decrease in the incidence of the disease.

Of the organizations affected through cerebrospinal meningitis 29 were also affected with measles, 10 with mumps, 7 with pneumonia, 2 with influenza, while bronchitis was fairly prevalent throughout the camp.

While the removal of the healthy carrier is of great importance in controlling the spread of the disease, little definite knowledge has been gained as to the best methods for freeing the carrier of the meningococcus. The use of the dichloramine-T spray as well as other antiseptic solutions no doubt eliminates the organism for a time, so that it is impossible to detect them by culture. Sooner or later, however, when the preventive spraying is discontinued, the meningococcus may again be recovered from the nasopharynx by culture. The following culture record illustrates this:

F. L. K., Pvt., Hdq. Co., 318 F. A., Dec. 24, 1917, pos. Dec. 31, neg. Jan. 7, neg. released from carrier camp, found again pos. Feb. 13, 1918, and readmitted to carrier camp.

The duration of the period of incubation has been variously placed by the different observers. Wright placed this period from one to ten days, Sophian from one to five. Our observations are in accordance with those of Wright in the Glasgow epidemic, as the following cases indicate:

1. Z. L., Sgt., Hdq. Co., 323 Inf., worked in office with B. A. who was cultured, found positive Jan. 16, 1918, and removed to the carrier camp the same date. Z. was admitted to the Base Hosp. Jan. 25, diag. Fever undetermined. Diag. C. S. M. established Jan. 26. The period of incubation in this instance is nine days.

2. L. F. R., Pvt., Remount Depot, admitted to Hosp., Jan. 25, 1918, diagnosis influenza. On the same date diagnosis C. S. M. was established. This patient boasted of having slipped by the sentry for the purpose of visiting the 317 M. G. Bat., Co. B., about one week before his admission. On Jan. 20, McK. of the 317 M. G. B. Bat., Co. B, was admitted to the Base Hospital, diag. C. S. M. In this instance the period of incubation was about 5 days.

3. That the incubation period may be still shorter is indicated by the following case: C. T. B., Pvt., Co. 13, 156 D. B., cultured Jan. 11, found neg., recultured Jan. 13, a gram-neg. Diplococcus, morphologically meningococcus was isolated. The organism did not agglutinate. Jan. 14, C was admitted to the Base Hospital, diag. Fever undetermined. Diag. C. S. M. established Jan. 15, 1918. The probable period of incubation in this case is two days.

The onset of the symptoms probably marks the time when the meningococcus gains entrance into the system from the nasopharynx, where it has existed from time of infection.

The small percentage of carriers who contracted the disease raised the question as to their immunity to the organism they harbored. The problem had a wider application, for if there existed a demonstrable immune body in the serum of the carrier, an immunity might possibly be induced by the use of suspensions of killed meningococci in healthy individuals as a prophylactic measure.

It became apparent that results of the agglutination experiments depended in a large measure upon the strain of meningococci used. The suspensions of meningococci used for agglutinations were made from hemolyzed blood glucose agar slants. The growth was washed off with salt solution, the suspension filtered through gauze and placed in the incubator at 56 degrees C. for several hours to destroy the bacteriolytic ferment.

The agglutinations were also incubated at this temperature.

TABLE V.

Name	Dilution				Salt Sol.
	1:10	1:20	1:40	1:80	
1. W. B.	——	——	——	——	——
2. B. Th.	——	——	——	——	——
3. B. G. W.	——	——	——	——	——
4. G. H.	——	——	——	——	——
5. F. W.	——	——	——	——	——
6. F. J.	——	——	——	——	——
7. Max Chr.	——	——	——	——	——
8. B. W. G.	——	——	——	——	——
9. J. H.	——	——	——	——	——
10. McA. J. M.	——	——	——	——	——
11. F. C. F.	——	——	——	——	——
12. D. E.	——	——	——	——	——
13. Th. C.	——	——	——	——	——
Rockefeller Poly. S.....	++	++	++	++	——

The sera were obtained Jan. 16, 1918, from the carriers indicated above. The agglutination test was run Jan. 18, the sera having been kept in the refrigerator in the interim. Strain used is that of carrier No. 1 of this table, isolated from the naso-

pharynx. This carrier was isolated Dec. 24, 1917, and kept in the carrier camp on account of repeated positive cultures, for at least four months.

Six sera obtained from patients admitted to the base hospital for suspected meningitis gave also negative agglutination reaction.

In view of the negative findings in the above experiment strain No. 5 isolated from the spinal fluid Dec. 15, 1917, was chosen, because this strain had been under transplantation for one month.

Table 6 indicates the results of this experiment.

TABLE VI.

Name	Dilution of serum 1:10	1:20	1:40	Salt. Sol. Control
1. W. B.	—	—	—	—
2. B. Th.	—	—	—	—
3. B. G. W.	—	—	—	—
4. G. H.	—	—	—	—
5. F. W.	—	—	—	—
6. F. J.	—	—	—	—
7. M. Ch.	—	—	—	—
8. B. W.	—	—	—	—
9. J. H.	—	—	—	—
10. McA. J. M.	++	++	+	—
11. F. C. F.	++	++	+	—
12. D. E.	—	—	—	—
13. Th. C.	++	++	+	—
Rockefeller Poly. S.	++	++	++	—

The sera of the six admissions to the hospital giving negative reaction with the carrier strain B. W., gave the following reactions with strain No. 5:

Name	1:10	1:20	1:40	Control
1. McC. Ph.	++	++	+	—
2. H. C. J.	++	++	+	—
3. Brady	++	++	+	—
4. Berry	++	—	—	—
5. F.	++	++	+	—
6. P. A.	++	++	+	—

The agglutinations in table 6 were carried out Jan. 19, 1918, with the same sera as used for the previous experiment (Table 5). The positive findings in three of the carriers would indicate that the results obtainable are dependent on the strain of meningococcus used in preparing the suspension for agglutination.

In all 160 sera of carriers from the carrier camp were tested by agglutination with strains No. 5 and No. 168 obtained from the spinal fluid. Aside from the three positive agglutinations of Carriers Nos. 10, 11, 13 of Table 6 no additional sera of carriers agglutinated either of the above strains. In comparing our results with those of F. L. Gates,[3] it seems possible that our initial dilution of the sera (1:10) was too high.

To determine whether agglutinations occurred in the blood of cerebrospinal meningitis patients early in the disease blood was obtained from the following cases on admission and agglutinations carried out with Nos. 5 and 168.

TABLE VII.

	Name	Organization	Date of obtaining serum	1-22-18 Strain 168	1-24-18 Strain 5
1.	H. Thos. C.,	323 Inf., Supply Co....	1-19-18	0	0
2.	R. Jos.,	316 M. G. Bat., Co. B......	1-19-18	0	1:20
3.	B. B. L.,	324 Inf., Co. M...........	1-20-18	0	1:40
4.	S. McK.,	317 M. G. Bat., Co. B......	1-20-18	0	0
5.	C. Chas.,	156 D. B., Co. 19.........	1-20-18	1:320	1:320
6.	Th. R. L.,	156 D. B., Co. 20........	1-21-18	0	0
7.	A. J. H.,	317 F. A., Bat. D........	1-22-18	0	1:20
8.	L. M.,	306 Eng., Co. D.............	1-21-18	0	0
9.	H. F.,	322 Inf., Co. D..............	1-22-18	0	1:40
10.	D. S.,	156 D. B., Co. 23............	1-22-18	0	0
R. Polyv. S.				1:400+	1:400+

Patients 6 and 7 were carriers and were admitted to the hospital from the carrier camp on the dates their sera were taken. The sera of these patients gave no agglutination except in one instance (5), when strain 168 was used while agglutinins were demonstrable in one-half the number of sera used when a strain of meningococci was used which agglutinated readily. Aside from the polyvalent serum and salt solution controls a normal horse serum control was used in all agglutination experiments. Case 2, although giving an agglutination of 1:20 with strain No. 5, died from the disease. The lowest serum dilution employed was 1:10. It is probable that a greater number of sera might have given agglutination in still lower dilution.

In view of the findings in these cases on admission, it seemed advisable to determine whether the agglutinins increased during the course of the disease. The results of the agglutination on treated patients are indicated in Table 8.

3. Jour. of Exp. Med. Vol. VIII, No. 4, pp. 449-474. Oct. 1, 1918.

TABLE VIII.

	Initials	Organization	Date of admission to Hosp.	Diag. made in Lab.	No. of days in Hosp.	Jan. 19, 1918 strain No. B. W.	strain No. 5
1.	G. S.,	316 F. A., Bat. D.	12-24-17	12-25-17	26	1:40	1:40
2.	Th. F.,	156 D. B., Co. 13	12-11-17	12-11-17	39	1:20	1:40
3.	A. L.,	316 F. A., Bat. A.	12-14-17	12-15-17	36	0	1:40
4.	B. Th.,	371 Inf., Co. C.	12-16-17	12-16-17	34	0	1:40
5.	D. R.,	316 F. A., Bat. A.	1- 6-18	1- 6-18	13	0	1:40
6.	A. Cl.,	156 D. B., Co. 15	12-29-17	12-30-17	21	—	—

	Jan. 22, 1918 No. 168	Jan. 24 No. 5	Nature of Treatment.
1.	1:80	1:320	Intraspinally only.
2.	1:40	1:320	Intravenously, large amounts.
3.	1:320	1:160	Intraspinally only.
4.	1:40	1:320	Intraspinally only.
5.	0	1:320	Intravenously, large amounts.
6.	0	1:80	Intravenously, large amounts.

Cases 2, 5 and 6 received average or small amounts of antimeningococcic serum intraspinally. These patients were classified as severe cases of meningitis. The production of agglutinins in the blood of the patient appears to increase during the course of the disease. That this increase in agglutinins is not entirely due to the agglutinins introduced with the therapeutic serum demonstrated by the findings in the blood of patients 1, 3 and 4, who were treated intraspinally only, the amount of serum given hardly exceeding 40-50 c. cm. at any one treatment while patients 2, 5, and 6 received from 100-125 c.cm. of serum at one time. That the agglutinins are formed by the patient was brought out in the agglutination test with the serum of case 3 C. Ch. Pvt., 156 D. B., Co. 19, whose serum agglutinated both strains Nos. 5 and 168 in a dilution of 1:320. This patient, a meningitis suspect, received no serum treatment, because the laboratory findings of his spinal fluid were negative.

We were unable to demonstrate agglutinins in the spinal fluids of a large number of patients, both during their period of active treatment and after their spinal fluid had become free of organisms, and the pathological excess of cells.

Aside from a few cases of cerebrospinal meningitis among the civil population of Columbia, S. C., who for one reason or another could not be transferred to the Base Hospital, Camp Jackson, the majority of civilian patients received their initial treatment at Columbia and were then transferred to the Base Hospital. It is of interest to note that not all the civilian patients thus admitted suffered from cerebrospinal meningitis traceable to contact with enlisted personnel or the proximity of the camp to the city. For one of these, a negro, became ill with cerebrospinal meningitis while serving an extended term in jail; the other, Hayward Trezevérant, admitted Jan. 31, 1918, had never been near the camp and had just arrived from Ft. Mott.

The prophylactic measures taken by the Board of Health of the city consisted in the prompt isolation of all suspicious cases and those who had been in contact with them. The latter were cultured for meningococci of the nasopharynx and kept under quarantine until found free. Public schools, churches and places of popular amusement were closed and the use of street cars by children discouraged for about six weeks during the height of the epidemic. Throughout the epidemic there was the closest co-operation between the Division Surgeon and the health authorities of the city and State. The prophylactic measures agreed upon for those desiring to visit the city have been indicated in the earlier part of this communication.

I take pleasure in acknowledging the assistance given me by Drs. A. H. Bunce, G. C. Brunelle, J. S. Fleming, G. F. Klugh, E. H. McClean and A. V. Solomon, members of the laboratory staff, Base Hospital, Camp Jackson, S. C.

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 235, 1918.

(Reprinted from *The Journal of the American Medical Association*, Vol. 70, No. 10, March, 1918.)

CULTIVATION OF THE MENINGOCOCCUS INTRACELLULARIS (WEICHSELBAUM) FROM THE BLOOD.

PRELIMINARY REPORT.*

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The recent epidemic of meningitis at this camp afforded excellent opportunities for studying the disease, and brought out certain phases in its onset and course which, heretofore, have not received the attention that their importance warrants. The unusual severity with which the disease manifested itself no doubt accentuated those phases so that they stood out more prominently in its syndrome.

While it is generally accepted that the meningococcus is carried in the secretions of the nasopharynx of patients and contacts, its mode of reaching the sites of the body where its presence gives rise to the pathologic manifestations is as yet to be definitely settled. Whether the meningococcus passes directly to the nervous system by way of the lymphatics between the nasopharynx and the meninges, or indirectly by way of the blood, has not been established.

The nature of the onset and course of the disease in some

*From the pathologic laboratories, base hospital, Camp Jackson, S. C.

cases pointed to the probable existence of a systemic infection before the meninges were affected. The course of the disease seemed to divide itself into two periods, the premeningeal and the meningeal. The premeningeal was characterized by chills, severe toxemia, and petechiæ. The spinal fluid drawn during this interval was frequently clear, free from polymorphonuclear leukocytes, and contained few or no organisms. During this stage Noguchi's butyric acid test for globulins was either very faint or negative. Fehling's solution was reduced.

The progress of the disease in these cases, showing symptoms of a general toxemia previous to or coexistent with those of meningeal involvement, suggested that as in other infectious diseases, as typhoid and pneumonia, we were dealing with a bacteremia which in its later course involves the meninges.

That the meningococcus occasionally gets into the blood is borne out by the occurrence of lesions in various parts of the body from which we have recovered and identified the meningococcus. These lesions have always been regarded as late systemic manifestations of the disease; and the occurrence of the organism in the blood, even though it has frequently been recovered by blood culture, was considered as exceptional. The early appearance of these lesions and the characteristics of the disease mentioned above lead us to believe that it is essentially systemic, and caused us to advise the intravenous administration of serum, which in the hands of the clinicians has shown such promising results. An occasional blood culture taken in the course of the epidemic confirmed us in the belief that the organism, in the early stages of the disease, invades the blood; and as the stress of routine work permitted, further cultures were made.

While the series reported here is small (twenty-five cases), it is very suggestive. When it is considered that the meningococcus, under the most favorable circumstances, is an extremely difficult organism to grow artificially at first from the cerebrospinal fluid, and that a blood organism is highly parasitic, the percentage of positive cultures obtained would point to a much higher incidence than the actual figures would indicate.

As large amounts of a vigorous growth have to be transferred to obtain successful transplants, so a large quantity of blood is necessary for the inoculation of the first culture. In the milder

cases the number of micro-organisms even in a large amount of blood is necessarily small; for this reason it is in the relatively severe cases, in which the number of organisms is large, that the positive culture is obtained.

The fact that the organism has been isolated from lesions in which the meningococcus infection has not been suspected points to the probability that even in the milder cases the disease is general, rather than a local infection confined to the meninges.

Up to the present the stress of work has prevented us from taking more than a single culture from each patient, excepting in two cases, one of which was twice positive and the other twice negative. If repeated cultures were taken they would probably show an even higher percentage of positives.

At present a culture is taken as soon as the patient is admitted and the provisional diagnosis made, and before any serum has been given. A member of the laboratory staff is always on call at any hour to get the blood. At the same time sufficient blood is obtained for agglutination tests, the results of which will be reported later.

The occurrence of the meningococcus in other sites of the body has been pointed out. That this metastatic localization may follow the meningeal involvement must be granted; it is equally possible that the occurrence of the organism in other regions than the meninges is due to the systemic invasion occurring in the early stages of the disease.

REPORT OF CASES.

Case 1.—Private E. S., aged 26, admitted, Dec. 2, 1917, complained of sore throat of two days' duration. He began feeling chilly on the morning prior to admission. He complained of headache, and pains in his bones and muscles. The provisional diagnosis was acute pharyngitis. December 8, 40 c.c. of slightly cloudy fluid were removed by lumbar puncture. The fluid contained many pus cells and intracellular and extracellular gram-negative diplococci. December 19, the patient developed arthritis of both knees; 45 c.c. of gelatinous purulent fluid were removed from the left knee, and 40 c.c. of a similar fluid from the right knee joint. Bacteriologic examination of these fluids was negative. December 25, a second puncture of the knee joints revealed fluids of a character similar to that removed by the first aspiration. Jan. 6, 1918, the patient developed panophthalmitis of the left eye, which was enucleated. Smears from the interior of the eye revealed numerous gram-negative, intracellular and extracellular diplococci. January 8, the patient was discharged. The bacteriologic examination of the knee fluids gave a gram-negative diplococcus which was identified as a meningococcus of the regular type.[1]

1. Final identification of the meningococcus is made by agglutination with serums supplied by the Rockefeller Institute and the Department of Health of New York City. Types are determined by serums furnished by the Army Medical School.

TABLE 1.—CASES ON WHICH THIS REPORT IS BASED

	Case	Date	Initial Chill	Headache	Vomiting	Rash	Temperature on Admission	Cerebration	Kernig's Sign	Rigidity of Neck	Microscopic Examination of Spinal Fluid	Culture of Spinal Fluid*	Culture of Blood	Culture of Nasopharynx	Treatment Before Admission	Outcome of Case	No. of Times Spinal Fluid Was Cultivated
1.	R. S. B.	12/14/17	+	+	0	++	?	Cloudy	?	?	+	0	+	—	0	L	1
2.	W. B. W.	12/14/17	0	0	0	+	100.8	Unconscious	0	0	+	0	0	—	0	D	1
3.	J. D. D.	1/ 7/18	0	++	0	0	101.4	Dull	+	+	+	0	+	—	0	D	1
4.	P. B.	1/13/18	+	++	++	++	104.2	Stupor	+	+	+	+	+	—	0	D	1
5.	J. P. C.	1/14/18	0	+	0	+	100.0	Clear	+	+	+	+	+	—	0	D	2
6.	C. W. L.	1/15/18	0	+	+	0	104.0	Stupor	++	+	+	0	0	—	—	L	12
7.	O. J. B.	1/16/18	0	+	++	+	103.0	Normal	++	+	+	+	+	+	0	D	2
8.	A. P.	1/17/18	0	+	0	0	103.0	Unconscious	+	++	+	+	0	0	0	L	1
9.	B. L. B.	1/20/18	+	++	++	0	101.4	Normal	sl	sl	+	0	0	0	0	L	4
10.	H. A.	1/21/18	+	+	+	0	100.2	Clear	sl	sl	+	0	0	0	0	L	5
11.	M. L.	1/21/18	0	+	+	0	98.0	Conscious	++	+	+	+	+	0	0	L	2
12.	R. L. T.	1/21/18	+	+	+	+	105.0	Conscious	+	+	+	+	+	+	0	D	2
13.	A. S.	1/22/18	0	+	+	0	103.0	Unconscious	+	+	+	+	0	0	+	L	4
14.	H. C. L.	1/23/18	†	+	0	0	99.0	Conscious	sl	sl	+	0	0	0	0	L	4
15.	W. F. M.	1/23/18	+	++	0	++	105.0	Clear	+	+	+	+	+	0	0	L	3
16.	J. W. W.	1/23/18	+	+	+	+	103.0	Delirious	+	+	+	+	+	0	0	L	1
17.	D. W.	1/23/18	0	+	0	0	101.0	Prostrate	+	sl	+	+	0	0	0	L	2
18.	Lt. D.	1/24/18	0	+	+	?	98.6	Clear	0	0	+	+	0	0	0	D	2
19.	R. F.	1/25/18	+	++	+	0	100.0	Unconscious	+	+	+	0	0	0	+	L	4
20.	H. F. J.	1/25/18	0	+	+	0	101.0	Cloudy	+	+	+	0	0	0	0	L	6
21.	L. C.	1/26/18	0	+	+	0	99.2	Dull	++	+	+	0	0	0	0	L	4
22.	R. B.	1/26/18	+	+	+	+	?	Delirious	++	+	+	0	0	0	0	L	4
23.	D. S.	1/28/18	0	0	0	++	102.0	Unconscious	+	+	+	0	+	0	0	L	2
24.	L. A. H.	1/28/18	+	+	+	0	102.0	....	sl	+	0	+	0	0	0	L	3
25.	J. W. J.	1/28/18	+	+	+	0	100.0	Clear	+	+	+	0	0	0	0	L	4

* Number of spinal fluid cultures positive, 12, or 48 per cent. The comparatively low percentage of positive cultures from the spinal fluids is probably due to the fact that the spinal fluids became chilled during the interval, as very often an hour or more elapsed between the time of puncture and final delivery at the laboratory; while on the other hand, the blood cultures were carefully guarded against chilling.

† Sweats.

CASE 2.—Private J. M. F., aged 25, admitted, Jan. 5, 1918, was in very poor condition; there were marked tremor, and cyanosis of the lips and finger tips. The provisional diagnosis was bronchitis following measles. January 11, broncho-pneumonia developed. The patient died, January 15. Cultures of pus found at necropsy in the sphenoid sinus gave a gram-negative diplococcus which was identified as the meningococcus of the regular type.

CASE 3.—Private S. R., aged 23, admitted, Nov. 15, 1917, complained of cold and pain in the chest, and pain in the eyes and the back. The provisional diagnosis was measles. November 24, bronchitis developed on the left side. November 28, there was pleurisy on the left side. Next day a diagnosis of broncho-pneumonia was made. Jan. 15, 1918, an intercostal incision for left empyema was made, and a large amount of slightly cloudy serous fluid removed, which was sent to the laboratory for bacteriologic examination. A meningococcus of the regular type was isolated.

COMMENT.

While Case 1 permits the conclusion of metastatic involvement during the course of the disease, Cases 2 and 3 indicate that the meningococcus infection may occur as a generalized bacteremia without causing noticeable meningeal symptoms.

The mediums as well as the method of carrying on this work are given in greater detail, because they can be followed under field conditions, and furthermore, on them depend in a large measure the results obtained in this series of cases.

Mediums Employed.—From 80 to 100 c.c. of meat infusion bouillon, salt free, are sterilized in 6-ounce medicine bottles. The advantages in the use of these containers lie in the facts that they are easily obtained, and that the narrow mouth reduces the chances of contamination.

To this bouillon are added 1 per cent. glucose and 10 per cent. serum water. This medium is stored in the incubator to be thoroughly warm at the time of inoculation. Two bottles are inoculated by the placing of from 15 to 20 c.c. of the patient's blood in each. A culture is made at the same time from the patient's nasopharynx on a glucose-blood-agar plate. The plates are examined the following day for suspicious looking colonies, and these are transferred to a glucose-blood-agar slant for identification.

The blood cultures are incubated at 37° C. and subcultures are made at twenty-four hour intervals on 1 per cent. glucose-blood-agar plates. Positive cultures are obtained in from forty-eight to ninety-six hours. Negative cultures are kept under observation for an additional five days before being discarded.

Appearance of Positive Bouillon Culture.—Red blood cells lie free on the bottom of the flask unchanged. The supernatant fluid

TABLE 2.—AGGLUTINATION OF STRAINS OF MENINGOCOCCUS

Case	Serum	1:50	1:100	1:200	1:400	1:800	N. II	N. S	1:1,600	Remarks
1*	..	..	..	..	..	..	..	..	..	Meningococcus from blood culture not typed
2*	..	..	..	..	..	..	..	..	..	
3 ([illegible])	R. P.†	++	++	++	+	±	—	—	—	Cerebrospinal fluid culture
	1	++	++	++	+	±	—	—	—	
	10	+	+	±	—	—		—	—	
	30	++	+	±	—	—	—	—	—	
	60	+	±	—	—	—	—	—	—	
4 ([illegible])	R. P.	++	++	+	±	—	—	—	—	Cerebrospinal fluid culture
	1	++	++	++	+	±	—	—	—	
	10	++	+	±	—	—	—	—	—	
	30	++	+	+	±	—	—	—	—	
	60	+	—	—	—	—	—	—	—	
([illegible])	R. P.	++	++	+	±	—	—	—	—	Blood culture
	1	++	++	++	+	±	—	—	—	
	10	++	+	±	—	—	—	—	—	
	30	++	+	+	±	—	—	—	—	
	60	±	—	—	—	—	—	—	—	
5 ([illegible])	R. P.	++	+	±	—	—	—	—	—	Cerebrospinal fluid culture
	1	++	++	+	±	—	—	—	—	
	10	++	+	±	—	—	—	—	—	
	30	++	+	+	±	—	—	—	—	
	60	+	—	—	—	—	—	—	—	
([illegible])	R. P.	++	++	+	±	—	—	—	—	Blood culture
	1	++	++	++	++	+	—	—	—	
	10	+	±	—	—	—	—	—	—	
	30	+	+	±	—	—	—	—	—	
	60	—	—	—	—	—	—	—	—	
6	..	..	..	..	..	..	..	..	..	Culture cerebrospinal fluid and blood negative
7 ([illegible])	R. P.	++	++	+	—	—	—	—	—	Nasopharynx culture
	1	++	++	++	+	±	—	—	—	
	10	++	+	±	—	—	—	—	—	
	30	++	—	—	—	—	—	—	—	
	60	++	—	—	—	—	—	—	—	
([illegible])	R. P.	++	++	—	—	—	—	—	—	Cerebrospinal fluid culture
	1	++	++	++	++	+	—	—	—	
	10	++	±	—	—	—	—	—	—	
	30	++	+	—	—	—	—	—	—	
	60	±	—	—	—	—	—	—	—	
([illegible])	R. P.	++	++	+	±	—	—	—	—	Blood culture
	1	++	++	++	±	—	—	—	—	
	10		—	—	—	—	—		—	
	30	++	±	—	—	—	—	—	—	
	60	—	—	—	—	—	—	—	—	
8 ([illegible])	R. P.	++	++	++	±	—	—	—	—	Cerebrospinal fluid culture
	1	++	++	++	++	+	±	—	—	
	10	±	±	—	—	—	—	—	—	
	30	++	++		±	—				
	60	+	±	—	—	—	—	—	—	
9	..	..	..	..	..	..	..	..	..	Culture cerebrospinal fluid and blood negative
10	..	..	..	..	..	..	..	..	..	Culture cerebrospinal fluid and blood negative
11 ([illegible])	R. P.	++	++	+	±	—	—	—	—	Cerebrospinal fluid culture
	4	++	++	++	+	—	—	—	—	
	10	—			—	—	—	—	—	
	30	+	—	—	—	—	—	—	—	
	60	±	—	—	—	—	—	—	—	
(282)	R. P.	++	++	+	±	—	—	—	—	Blood culture
	4	++	++	++	±	—	—	—	—	
	10	—	—	—	—	—	—	—	—	
	30	±	—	—	—	—	—	—	—	
	60	—	—	—	—	—	—	—	—	
12 (282)	R. P.	++	++	++	++	++	—	—	—	Nasopharyngeal culture
	4	++	++	++	++	±	—	—	—	
	10	++	+	±	—	—	—	—	—	
	30	++	++	++	+	±	—	—	—	
	60	++	++	±	—	—	—	—	—	
(277)	R. P.	++	++	++	++	+	—	—	—	Cerebrospinal fluid culture
	4	++	++	++	++	±	—	—	—	
	10	+	±	—	—	—	—	—	—	
	30	++	++	++	±	—	—	—	—	
	60	++	+	±	—	—	—	—	—	
(284)	R. P.	++	++	++	+	±	—	—	—	Blood culture
	4	++	++	++	++	+	—	—	—	
	10	++	++	+	±	—	—	—	—	
	30	++	++	+	±	—	—	—	—	
	60	++	++	++	—	—	—	—	—	
13 (297)	R. P.	++	++	++	±	—	—	—	—	Cerebrospinal fluid culture
	4	++	++	++	+	±	—	—	—	
	10	++	±	—	—	—	—	—	—	
	30	+	±	—	—	—	—	—	—	
	60	++	+	±	—	—	—	—	—	
14	..	..	..	..	..	..	..	..	..	Culture cerebrospinal fluid and blood negative
15	R. P.	++	++	++	±	—	—	—	—	Cerebrospinal fluid culture
	4	++	++	++	+	±	—		—	
	10		—	—	—	—	—	—	—	
	30	++	±	—	—	—	—	—	—	
	60	++	+	—	—	—	—	—	—	
16 (278)	R. P.	++	++	+	±	—	—	—	—	Cerebrospinal fluid culture
	4	++	++	++	+	±	—	—	—	
	10	++	++	±	—	—	—	—	—	
	30	++	++	—	—	—	—	—	—	
	60	++	++	±	—	—	—	—	—	
(285)	R. P.	++	++	++	±	—	—	—	—	Blood culture
	4	++	++	++	+	±	—	—	—	
	10	++	++	±	—	—	—	—	—	
	30	++	+	±	—	—	—	—	—	
	60	++	++	±	—	—	—	—	—	
17 ([illegible])	R. P.	++	++	+	±	—	—	—	—	Cerebrospinal fluid culture
	4	++	++	++	+	±	—	—	—	
	10	+	—	—	—	—	—	—	—	
	30	++	++	±	—	—	—	—	—	
	60	++	+	—	—	—	—	—	—	
18 ([illegible])	R. P.	++	++	++	+	±	—	—	—	Cerebrospinal fluid culture
	4	++	++	++	++	±	—	—	—	
	10	++	±	—	—	—	—	—	—	
	30	++	++	+	±	—	—	—	—	
	60	++	+	—		—	—	—	—	
19	..	..	..	..	..	..	..	..	..	Culture cerebrospinal fluid and blood negative
20	..	..	..	..		..	..	..	..	Culture cerebrospinal fluid and blood negative
21	..	..	..	..	..	..	..	..	..	Culture cerebrospinal fluid and blood negative
22	..	..	..	..	..	..	..	..	..	Culture cerebrospinal fluid and blood negative
23 (304)	R. P.	++	++	+	±	—	—	—	—	Blood culture
	4	++	++	++	+	—	—	—	—	
	10	—	—	—	—	—	—	—	—	
	30	++	±	—	—	—	—	—	—	
	60	++	++	+	—	—	—	—	—	
24 ([illegible])	R. P.	++	++	++	+	±	—	—	—	Cerebrospinal fluid culture
	4	++	++	+	+	—	—	—	—	
	10	+	±	—	—	—	—	—	—	
	30	++	++	—	—	—	—	—	—	
	60	++	—	—	—	—	—	—	—	
25	..	..	..	..	..	..	..	..	..	Culture cerebrospinal fluid and blood negative

* Blood culture negative.

† R. P. in this table refers to Rockefeller polyvalent serum. Nos. 1, 10, 30 and 60 are the Rockefeller type serums as furnished by the Army Medical School. These were furnished in two lots. The No. 60 of one lot has a cross agglutination titer of 1:400 with No. 10. The No. 4 serum was kindly furnished by Capt. Harold L. Amoss, M. R. C. Nos. 1 and 4 are representative of the normal or regular type. Nos. 10 and 30 are intermediates. No. 60 is a parameningococcus. It will be noted that these cultures belong to the normal or regular type.

is slightly cloudy and has a faintly greenish opalescence. As the culture ages, the color of the medium deepens.

Method of Making Subcultures.—From the bottom of the flask just above the sedimented blood cells, about 1 c.c. of the culture is withdrawn, poured over each of a series of 1 per cent. glucose-blood-agar plates, and incubated with the cover up. (A 2 per cent. agar plus 0.2 phenolphthalein, to which 1 per cent. of glucose and 15 per cent. of laked human blood is added.)

In from sixteen to twenty-four hours, the liquid has evaporated from the plate, and the growth appears as either a grayish-white gelatinous coat or as discrete colonies. At the time of transfer from the bouillon culture to the plates, a transfer is also made to a slant of the same medium by allowing 0.2 c.c. of the bouillon culture to run over the slant. This frequently gives sufficient growth for agglutinating purposes.

The colonies on an eighteen to twenty-four hour growth, with the employment of the foregoing medium, are from 0.5 to 3 mm. in diameter, and are round, with an even, circular, smooth edge and a smooth surface. By reflected light the colony appears gray and glistening, with a soft, velvety tone. By transmitted light it has the general appearance of a drop of honey, though faintly hazy. When touched procedure frequently gives sufficient growth for agglutinating purposes.

SUMMARY.

In this series of twenty-five cases, we have included only those cases in which, in the spinal fluid, organisms were found having the morphology of the meningococcus.

The findings in the three early cases induced us to make routine cultures.

The remaining twenty-two cultures were taken in consecutive cases, giving 36.3 per cent. of positives.

CONCLUSIONS.

1. Systemic infection by the meningococcus is more frequent than previously suspected.
2. This systemic infection may occur without appreciable or with no meningeal localization.
3. Systemic infection may be previous to, or coexistent with, meningeal involvement.
4. Consequently the intravenous administration of antimeningococcic serum is rational, and is indicated in conjunction with the intraspinal treatment.

For the histories quoted in this communication, we are indebted to Major Herrick, M. R. C., chief of the medical service.

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 236, 1919.

(Reprinted from the *Journal of the American Veterinary Medical Association,* Vol. LVI, N. S. Vol. 9, No. 3, December, 1919.)

STUDIES ON ANTHELMINTICS.

VI. Tests of the Administration of Anthelmintics in Enteric-Coated Soft Gelatin (Soluble Elastic) Capsules.

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Anthelmintics intended to remove worms from the small intestine must first pass the stomach, and we know that a certain amount of the anthelmintic is absorbed by the stomach, thereby adding to the systemic toxic effects, in addition to diminishing the amount of anthelmintic available against the worms in the small intestine. Furthermore, the anthelmintic undergoes dilution by the fluids and ingesta in the stomach, which dilution may reach a point where it will perceptibly diminish the efficacy of the drug and may render it entirely ineffective.

To avoid the above objectionable effects from the passage of anthelmintics through the stomach, it would appear to be the logical thing to enclose the drug in an enteric coat, so that the drug would be released in the intestine, thereby avoiding absorption and dilution in the stomach. In actual practice, enteric coats are not entirely satisfactory affairs. A number of substances which are comparatively insoluble in the acid gastric juice are used, such as talc-shellac, phenyl salicylate, keratin, and formalin-hardened gelatin, but there are objections of one sort and another to these. In our experience, talc-shellac coatings will open in the stomach at times or fail to open in the upper portion of the small intestine at times, and the formalin-hardened gelatin gets progressively harder with age and ultimately fails to open at all, passing the entire digestive tract.

Tests of soft capsules containing oil of chenopodium and coated with the talc-shellac preparation gave entirely satisfactory anthelmintic results. The dose used was that given by the writer (Hall, 1917) in a previous paper—5 minims to dogs weighing 10 pounds

*Resigned March 27, 1919.

or less, 10 minims to dogs weighing 10 to 20 pounds, 15 minims to dogs weighing 20 to 30 pounds, and 20 minims to dogs weighing over 30 pounds. In one or two instances where dogs weighed close to the upper limit of the 10-pound range given here, the additional 5 minims of the range above was given. The tests are given in the following table:

Dog No.	Wt. Kilos	Dose.	Worms passed.				Postmortem.					Ascaricidal efficacy, per cent.
			Ascarids.	Hookworms.	Whipworms.	Tapeworms.	Day.	Ascarids.	Hookworms.	Whipworms.	Tapeworms.	
65	14	20 v	2	0	0	0	4th	0	0	0	0	100
72	13	20 v*	5	0	0	0	6th	0	0	0	0	100
73	16	20	0	0	0	0	4th	0	0	10	0	...
74	6	10	3	0	0	0	4th	0	0	0	50	100
75	11	15	2	0	0	0	4th	0	0	0	8	100
76	7.5	10	3	0	0	0	4th	0	1	18	1	100
77	10	15	22	0	1	0	4th	0	0	22	76	100
78	12	15	6	0	0	0	4th	0	0	0	65	100
79	9	15 v	5	0	0	0	4th	0	0	0	22	100
80	7	10	6	0	0	0	4th	0	0	4	14	100
81	10	15	1	0	0	0	4th	0	0	0	0	100
82	10	15	108	4	0	0	4th	0	5	0	0	100
84	8	10	0	0	0	0	3rd	0	0	7	0	...
86	15	20 v	2	0	0	0	9th	0	0	0	0	100
91	7	10	4	0	0	0	6th	0	0	0	15	100
92	10	15	13	0	0	0	8th	0	0	1	1	100

v vomited capsules   * received 45 mils castor oil.

There are 16 dogs in the above experiment. The weight is given in kilos (1 kilo = 2.2 pounds). The dose is given in minims, and in all cases was followed immediately by 30 mils of castor oil, except in the case of Dog No. 72, where it was preceded by 15 mils of castor oil and followed by 30 mils additional, 15 minutes after giving the capsules. The 4 dogs marked *v* vomited their capsules, opened, which indicates that these capsules may open in the stomach at times, though the moistened capsule may have opened in the dog's esophagus during vomition or may have been broken by the teeth at this time; the anthelmintic efficacy was not noticeably impaired by the opening of the capsules, apparently. The dogs averaged a little less than 10 kilos in weight; Hall and Wigdor (1918) have shown that 10 kilos is about the average weight for dogs. They received an average dose of a little less than 18 minims, or a little more than 1 mil, which is close

to the therapeutic dose of 0.1 mil per kilo, which the writer (Hall, 1917) has established experimentally. Two dogs were not infested with ascarids, which is taken by the writer as the test worm for this anthelmintic. The 14 infested dogs had a total of 182 ascarids, all of which were removed, giving an efficacy of 100 per cent. Of a total of 10 hookworms, 4 were removed, or 40 per cent. Of a total of 63 whipworms, only 1 was removed, a total of less than 2 per cent. Of 252 tapeworms, none were removed, an efficacy of 0 per cent.

A series of tests were made with soft capsules containing 5 minims of oil of chenopodium and 10 minims of chloroform each, these capsules being treated to form an enteric coat of the gelatin by leaving them in 1 per cent formaldehyde for a half minute to a minute and then allowing at least two weeks to elapse before using. The capsules used were given their enteric coating by Wilbur L. Scoville and were less than a month old when used. After a year to a year and a half, according to Scoville, such capsules become so insoluble that they pass the digestive tract unopened.

In our series of tests we were unfortunate in being unable to obtain enough dogs infested with ascarids or hookworms to give the best sort of test, only 3 dogs out of 14 having ascarids and only one having hookworms. The tests were as follows:

Dog No.	Wt. Kilos.	Dose.	Worms passed.				Postmortem.					Ascaricidal efficacy, per cent.
			Ascarids.	Hookworms.	Whipworms.	Tapeworms.	Day.	Ascarids.	Hookworms.	Whipworms.	Tapeworms.	
98	10	3v	0	0	0	0	4th	0	0	1	371	...
93	8	2	0	0	4	0	11th	0	2	7	0	...
94	10	3	0	0	0	0	11th	0	0	125	0	...
99	8.5	2v	0	0	0	2	4th	0	0	2	0	...
100	11.5	3v*	0	0	0	0	5th	0	0	0	27	...
101	9	3v*	0	0	0	0	5th	0	0	0	0	...
102	7	2v*	0	0	4	0	5th	0	0	0	0	...
103	7	2	0	0	0	0	5th	0	0	0	0	...
104	11	3	4	0	0	0	5th	0	0	0	9	100
106	9.5	3v	0	0	0	0	12th	8	0	0	1	0
107	19.5	3	0	0	0	0	11th	0	0	0	10	...
109	8	2v	7	0	0	0	14th	5†	0	0	0	†58
112	9.25	3v	0	0	0	0	5th	0	0	0	56	...
113	8	2v	0	0	0	0	5th	0	0	0	0	...

v vomited after treatment. * given more than 30 mils castor oil with treatment. † ascarids in stomach.

The weight is given here in kilos. The dose is given in number of capsules, each capsule containing 5 minims of oil of chenopodium and 10 minims of chloroform, as noted above. The capsules were followed immediately with 30 mils of castor oil, except in the cases noted above, where larger doses were given. Eight of the 11 dogs vomited after treatment, bringing up the capsule in some cases. In one of these cases, Dog 106, where vomiting occurred, though no capsules were found in the vomitus, the treatment was an entire failure against ascarids. These dogs averaged a little less than 10 kilos in weight and received on an average about 13 minims of oil of chenopodium and 26 minims of chloroform. There were ascarids present in 3 dogs: The treatment removed all of these in one case, none of them in another case, where vomition occurred, and 58 per cent in another case (Dog 109). In this last case, the dog had 1 ascarid in the small intestine and 4 in the stomach. This develops one objection to the use of enteric-coated preparations for use against ascarids. These worms are notorious for their wandering habits, and the stomach is a favored site for wandering, especially in puppies. Obviously an enteric preparation that passes the stomach unopened will fail to remove ascarids located in the stomach.

While there is something fascinating about the idea of exploding an anthelmintic bombshell in the midst of the worms in the small intestine, there are certain difficulties in the way of a practical application. For one thing, the shell may pass by a number of worms in the duodenum and upper jejunum before exploding, and it is impossible to time the shells for various ranges and bracket the initial shots. It seems likely that the capsule would shove before it such worms as ascarids, especially in masses, until it broke, but it would easily slip by such worms as hookworms or even small ascarids. The treatment failed to remove the 2 hookworms present. It removed 8 of 143 whipworms, a little over 5 per cent, and removed 2 of 476 tapeworms, or less than 1 per cent.

The results obtained by the use of formalin-gelatin enteric coats, enclosing a mixture of oil of chenopodium and chloroform, are not satisfactory. On the other hand, the results obtained by the use of the talc-shellac coat are unusually good, the minimum therapeutic dose removing all the ascarids present from 14 infested dogs. Whether this efficacy is to be attributed in any part to the anthelmintic coat is debatable.

Just how uncertain is the action of an enteric coat may be judged from the following experiments:

Dog No. 332, a mongrel weighing 14 kilos, was given three 10-minim soluble elastic gelatin capsules of oil of chenopodium, enteric coated, with the talc-shellac preparation, the capsules being followed immediately with 30 mils of castor oil. Two hours and 5 minutes later the dog was shot, and 4 minutes thereafter the stomach was opened. The three capsules were found in the stomach unopened; the castor oil had apparently gone on through the small intestine.

Dog No. 334, a wolfhound mongrel weighing 14.5 kilos, was given three similar capsules and the same amount of castor oil. Three hours later the dog was shot and the stomach opened. Two capsules were found in the stomach; the enteric coats were cracked, but the capsules were not opened. One capsule was found in the lower jejunum, similarly with a cracked enteric coat, but unopened. The castor oil was in the cecum and the large intestine.

It appears from the above that enteric-coated capsules may lie in the stomach for long periods, while the accompanying purgative passes out; that the enteric coats may break partly by digesting and partly, perhaps, from mere moistening, softening, and the mechanical effects of peristalsis; that the capsules may pass as far as the lower jejunum, and perhaps to the large intestine, without opening; and that of the same lot of capsules, simultaneously administered, some may do one thing and some another. With talc-shellac coats, the ascaricidal efficacy is unimpaired, while no conclusions can be safely drawn from the showing of 40 per cent efficacy against hookworms.

Zeigler (1917) has also investigated the possibilities of enteric-coated capsules of oil of chenopodium, from a physiological standpoint, and without reference to their anthelmintic value. He used hard capsules, filled with a hypodermic syringe and coated with salol by dipping in melted salol, drying, and repeating the process until a sufficiently thick coat had formed. He notes that as ordinarily given, dogs will show symptoms of chenopodium absorption within 10 minutes, and that with the enteric coats, symptoms—and presumably absorption—were delayed from 30 minutes to an hour and a half. Of 10 animals given twice the minimum lethal dose (the m. l. d. is 0.5 m. p. k.), only 2 died; some only showed vomit-

ing and salivation. Two animals were given this dose and chloroformed at the end of 2 hours. The capsules had dissolved and evidence of their solution was found in the intestine.

It will be noted that Zeigler's findings differ from those given in this paper. He finds enteric capsules dissolved in 2 hours; we found them still in the stomach in 2 hours, and in the stomach and in the ileum in 3 hours. Of course, the explanation for this may lie in the fact that he used a salol coat on a hard capsule, and we used a talc-shellac coat on a soft capsule.

Zeigler states that, "The animals were placed in metabolism cages and watched carefully to see that none of the capsules were vomited or passed in the stools." It is to be regretted that all the dogs in his experiments were not killed and examined post-mortem. It would be interesting to learn why double the m. l. d. of this drug failed to kill or even produce marked symptoms in these dogs. Apparently it is due to the slight absorption of the drug in the intestine, but if this is the case, why did 2 of the dogs die? Did the capsules, perhaps, open in the stomach in these 2 cases? In this connection, Zeigler says: "The absorption is more rapid from the stomach than from the intestines." On the other hand, Salant and Livingston (1915) found that several hours may elapse before evidence of absorption into the circulation could be obtained when the oil was introduced into the stomach of animals, in which the pylorus had been previously ligated, whereas absorption from the duodenum was very rapid. In our own experience, we find that there is considerable variation in the case of individual dogs, but certainly there is a very rapid gastric absorption in most cases, the onset of symptoms following the administration of the drug very promptly. On the other hand, some dogs show little evidence of the presence of the drug at any time. Doubtless the conditions which have been mentioned by Salant and his collaborator enter into these cases—presence of hunger peristalsis, amount of fasting, etc. In our experience, the simultaneous administration of olive oil prolongs the period in which the castor oil remains in the stomach and increases the gastric absorption and production of gastric lesions.

Regardless of the fact in this case, and both findings might be right for the animals experimented on, the writer believes that less is to be expected from enteric-coated capsules which will allow

of chenopodium absorption only in the intestines than from the use of such purgatives as castor oil, which will distribute the absorption, and the ensuing shock and insult to the mucosa, over as wide a surface as possible and simultaneously prolong the period of absorption and allow the patient a longer period in which to dispose of the toxic elements absorbed. Zeigler concludes: "Just what effect this delayed absorption of the oil when administered in enteric capsules would have upon its vermicide effect I am unable to say at this time, but certainly I believe that the most rational method of administering the drug in hookworm disease would be in this manner." The writer cannot regard this entirely reasonable theory as sufficiently established by the available facts. The anthelmintic efficacy with the talc-shellac coats was well maintained, so far as ascarids are concerned, but even these capsules went at times to the ileum without opening, and the efficacy of the formol-hardened capsules was impaired. From the available facts, the writer is of the opinion that enteric coats add to the uncertainties of the action of anthelmintics, though more facts would be welcome.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 237, 1919.

(Reprinted from the *Journal of the American Pharmaceutical Association,* Vol. VIII, No. 12, December, 1919.)

CHLORETONE WATER: A NEW PRESERVATIVE OF BIOLOGICAL SPECIMENS.

By Oliver Atkins Farwell

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During the early part of this summer (1919) it was suggested to me by Dr. T. B. Aldrich to try out chloretone water as a vehicle in which to preserve vegetable material for permanent biological exhibits and as a means of keeping in a fresh condition vegetable material designed for early laboratory work. He told me that he had animal organs in chloretone water that had been kept for several years, and that they were apparently in good condition. He saw no reason why vegetable matter would not be equally well preserved. I therefore carried chloretone water on my botanical excursions, and collected various plants, such as Green Algæ, Water Lily stems, etc., and put them in the chloretone water. Plasmolosis has not occurred in any of the plants collected, which included both aquatic and terrestrial plants. It acts as a good fixing agent for filamentous algæ, but I have not tested it out along these lines on denser tissues or structures. It gives promise of being as good a fixing agent if not better than any now in use, and bids fair to outrival alcohol as a general preservative in both cheapness and efficiency. It kills all but the most resistant spore-bearing bacteria, so that no growth occurs in any media that contains a small quantity of chloretone.

A few grammes inclosed in a collector's bottle permanently quieted small moths in less than one minute, and a large blue-bottle fly in two minutes. It is therefore equal to chloroform in efficiency for the entomological collector. If a few grammes were placed in the bottom of the collector's bottle and held in place beneath a pledget of cotton batting, they probably would last throughout a whole season, or several seasons, provided the

bottle were not left uncorked unnecessarily, and possibly prove to be cheaper than chloroform. An aqueous solution from $\frac{1}{4}$ to $\frac{1}{2}$ of 1 per cent might answer their purpose equally well and be much cheaper in the bargain. Flies and yellow jackets after remaining for two months in a saturated solution had not lost their colors. Chloretone is soluble in water to the extent of from 0.5-0.8 per cent. A saturated aqueous solution of chloretone might cost anywhere from 1 to $1\frac{1}{2}$ dollars a gallon, depending largely on where the chloretone was procured. Samples of the fleshy Purslane (Portulaca oleracea) kept in an aqueous solution of 0.25 per cent strength have shown no signs of degeneration. Potamogetons taken from the Raisin river and corked up in a bottle full of the river water had, in the course of two months, largely become disorganized and much of it had disintegrated to such an extent as to have been transformed into sediment. It may be worthy of notice to state here, as an illustration of the tenacity of life under unfavorable conditions, that a single frond of *Spirodela* accidentally collected along with the Potamogetons had continued to grow and had increased to 4 fronds during the same length of time, and that the terminal point of one of the Potamogetons produced a new growth exceeding 3 inches in length. I have detected no changes of any kind in the organization of any of the plants preserved in chloretone water. They will, however, lose their color.

Laboratory trials and tests will fix the strength of the solution necessary for its varied uses. If the weaker solution is sufficient for killing and fixing, its cheapness will be about on a par with that of the chromacetic acid combination, but it will be much more satisfactory as a general fixing agent, as it will save much time now lost in the lengthy washing process necessary when the chromacetic fixer is employed. I strongly recommend to the scientific world, and especially to histological and morphological investigators, the use of an aqueous solution of chloretone for a thorough investigation as a substitute for alcohol as a preservative, and as a killing and fixing agent.

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 238, 1920.

(Reprinted from the *Journal of the American Veterinary Medical Association*, Vol. LVI, N. S. Vol. 9, No. 4, January, 1920.)

STUDIES ON ANTHELMINTICS.

VII. A Comparison of Castor Oil and Other Purgatives in Connection With the Administration of Some Anthelmintics.

By Maurice C. Hall, Ph. D., D. V. M.,[1] and Meyer Wigdor, M. A.

(Medical Research Laboratories, Parke, Davis & Company, Detroit, Mich.)

The question as to the best purgative for use in connection with the administration of oil of chenopodium is an unsettled one, but the two drugs most used are castor oil and magnesium sulphate or Epsom salts.

The Third Annual Report of the Rockefeller Foundation, dated January, 1917, says (p. 191), in regard to hookworm work in Trinidad: "It was necessary to use magnesium sulphate instead of castor oil, because in Trinidad there is an exceedingly strong local prejudice against the latter, and it is practically impossible to induce persons of the lower class to take it." Darling, Barber, and Hacker (1918) state: "We divided the squads of men into halves. Those on one side of the ward were given magnesium sulphate; those on the other side were given castor oil. The castor oil squad always showed the greater number of cases of dizziness and deafness; most of the cases of inability to rise and walk occurred in this group. The urine of patients taking castor oil contained much heavier precipitates when tested for oleoresin than those that had taken magnesium sulphate. Dizziness and muscular incoördination were less with magnesium sulphate than with castor oil, compound mixture of senna, or calomel." [In this connection, it is worth noting that Dr. David E. Buckingham, of Washington, D. C., has called the attention of one of us (Hall) to cases in his practice where deafness in dogs has followed the use

[1]Resigned March 27, 1919.

of chenopodium with a delay of 12 hours or so in administering castor oil.]

On the other hand, the Third Annual Report of the Rockefeller Foundation, quoted above, notes (p. 193) the following case: "The most serious mishap was collapse in a child of three, on whom the second dose of magnesium sulphate had no effect. She recovered after a dose of castor oil." Heiser (1917) reports the death of 2 children in Ceylon, where magnesium sulphate was used, and in comparing the results here with results in Sumatra, where there were no deaths in 300,000 treatments with chenopodium, he notes these differences in mode of administration: In Sumatra there were no dietary restrictions, no preliminary purgation, castor oil was used instead of magnesium sulphate, and the chenopodium was given in hard capsules. By way of comment, we may say that lack of dietary restrictions probably adds to a patient's safety, as a rule, but also commonly diminishes the anthelmintic efficacy; preliminary purgation has been abandoned by us as a routine procedure, as it seems to be unnecessary; of the 2 deaths cited by Heiser, 1 was a case where soft capsules were used, and 1 a case where no capsules of any sort were used; and the use of magnesium sulphate in the cases which died is apparently the only difference of importance. At that, there is the possibility of idiosyncratic intolerance to chenopodium or the presence of contraindications to the use of chenopodium in the cases where deaths occurred.

Fixed oils have been found of value in preventing chenopodium poisoning by Salant and Nelson (1915). Hall and Foster (1917, 1918) had excellent results with castor oil. In our experience—and we have here the data on 220 dogs which have been treated with chenopodium—the simultaneous administration of an ounce or two of castor oil with chenopodium gives very excellent results—good purgation and a high degree of protection against the gastro-intestinal irritation and the toxic effects. Some experiments indicating the protective action of castor oil in cases where double the minimum lethal dose had been given were published by Hall (1918) in Hall and Hamilton's (1918) paper on constituents of chenopodium. Those experiments showed the following results where 4 dogs were given 1.0 m. p. k. (mil per kilo) of chenopodium or a distillation product of chenopodium: One dog was given 15 mils of castor oil before treatment, 15 mils more with a cheno-

podium constituent, and 30 mils after the chenopodium, and was killed the sixth day after treatment. That the castor oil is protective, and not merely purgative, is shown by the fact that this dog passed no feces the first day after its treatment with double the lethal dose. A second dog was given chenopodium with the same amounts of castor oil in the same way, and was killed the sixth day after treatment. The protective action of the castor oil is again shown here by the fact that the dog passed no feces the first two days after treatment; the animal was then given another dose of 30 mils of castor oil. The other 2 dogs received the same amounts of chenopodium or chenopodium constituent in 50 mils of liquid petrolatum, and died the day after treatment, or the following day. A number of experiments show that liquid petrolatum diminishes the anthelmintic efficacy of oil of chenopodium and furnishes inadequate protection against toxic effects.

The protective action of castor oil against the toxic effects of oleoresin of male-fern have been pointed out in another paper by Hall (1918). Of 5 dogs receiving 20 mils of oleoresin of male-fern, a lethal dose, 1 dog received no purgative and died the sixth night after treatment; 2 received 60 mils of castor oil and were killed the sixth and eighth days after treatment; 1 received 30 mils of castor oil and was killed the eighth day after treatment; 1 received 6 grains of calomel and was killed the ninth day after treatment. To these records we may add the case of dog No. 180, a bulldog weighing 9 kilos, which received 20 mils of oleoresin of male-fern and 6 grains of calomel, and was killed 8 days afterward. The dogs that were killed had recovered from the treatment and were in good physical condition. These experiments show that one essential in the administration of male-fern is purgation. So far from castor oil increasing the absorption of male-fern and making it more dangerous, it will save animals that have been given a lethal dose, as will calomel.

In the same paper, Hall (1918) notes that a little more than the lethal dose of 0.5 m. p. k. of chenopodium, administered to a dog with an accompanying 100 mils of olive oil, and followed by 50 mils more 2½ hours later, resulted fatally in less than 24 hours. A number of other experiments with sub-lethal doses show that olive oil administered with oil of chenopodium affords little protection from toxic effects, hastens gastric absorption, and

delays the passage of the drug from the stomach, with the resultant production of severe gastric lesions.

In the following experiments each of 4 dogs was given a lethal dose of 0.6 m. p. k. of oil of chenopodium. One hour later 2 dogs were each given 60 mils of castor oil and 2 dogs were each given 15 grams of magnesium sulphate. The results were as follows:

Dog No. 287, a hound weighing 9.5 kilos, showed chenopodium poisoning about 15 minutes after treatment. The chenopodium was given in hard gelatin capsules. Dog staggered and slobbered profusely. Within a half-hour the animal had vomited and was lying down in its cage with feet outstretched. An hour after treatment an attempt was made to administer the magnesium sulphate, but the animal was in no condition to swallow properly and a considerable part of the salts never reached the stomach. The dog was found dead the next morning. This cannot be regarded as a test of magnesium sulphate, as conditions were unfavorable. The dog was a poor subject for treatment; the temperature was 102° F. before treatment, and the conjunctiva was inflamed, indicating distemper, and this was confirmed by post-mortem examination. Anthelmintics are contraindicated in distemper and other febrile conditions, even when given in therapeutic doses.

Dog No. 282, a spaniel weighing 11 kilos, was given the chenopodium in hard gelatin capsules. The dog showed no immediate effects, but in a half-hour it was staggering and slobbering. An hour after treatment it was lying down, unable to keep on its feet. It received the castor oil, and again lay down, setting its teeth in the wires of its cage. The next morning the dog was found dead; 60 mils of castor oil could not save this animal 1 hour after the administration of 0.6 m. p. k. of chenopodium.

Dog No. 281, a bull terrier weighing 9 kilos, was given the chenopodium without the use of capsules and lost over half of it. About a half-hour after dosing the dog vomited. At this time the dog was standing up and yelping noisily. An hour after dosing, the dog was staggering, and the magnesium sulphate was given at this time. The next morning the dog was lying on its side trembling. At 1:00 p. m. the dog was apparently unconscious, barking at intervals and moving the legs automatically and rather rapidly; temperature 96.3° F. At 2:00 p. m. the bark had changed to a whine. At 2:35 the dog was lying on its side, moving the legs in a

swimming stroke, and stopping and whining at intervals; temperature 97.4° F. The second day after treatment the dog was lying in its cage unconscious, kicking very feebly and breathing very slowly. Temperature, 94 at 8:00 a. m.; 94.2 at 11:00 a. m.; 94 at 4:00 p. m. The dog was not seen the next day (Sunday), but was found dead the following day. The magnesium sulphate could not save this animal one hour after a dose of 0.6 m. p. k. of oil of chenopodium. The dog was a young and active subject, but the eyes contained purulent matter before treatment, and the lungs showed a purulent bronchitis on post-mortem; the temperature before treatment was only 100.4° F., however.

Dog No. 286, a terrier weighing 8.5 kilos, was in good physical condition an hour after the chenopodium treatment; it had vomited and was trembling, but was still active. Gave castor oil. Temperature 101° before treatment. The next morning the dog was sitting down and seemed drowsy; in the afternoon the dog was standing, not trembling, and the temperature was still 101°. The following day the dog was standing up, but trembling, and had not yet eaten. The dog was not seen the third day after treatment (Sunday), but on the fourth day the dog had eaten and was in good condition. The dog was killed 18 days after treatment. The digestive tract was normal. The castor oil saved the life of this dog. Possibly the absorption of chenopodium was slow for some reason, as it seemed to be, so that the castor oil was in ample time to exert its protective action.

Unfortunately the above experiments do not give definite results on which to make a choice between castor oil and magnesium sulphate, and it is possible that no choice need be made. However, the experiments show that even after the lapse of an hour, following the ingestion of 0.6 m. p. k. of oil of chenopodium, which is more than the m. l. d., 60 mils of castor oil will save a dog's life at times and will fail to save it at times. No conclusions may be drawn from the experiment with dog No. 287; it confirms the conclusion that anthelmintics should not be given to animals with distemper. Dog No. 284 got less than a lethal dose of chenopodium, received the magnesium sulphate, and died, probably on the third day, but this dog was not in the best physical condition. The experiments, then, throw no light on the protective value of magnesium sulphate, but do confirm the value put on castor oil for use with chenopodium.

SUMMARY.

Experiments published in this paper and elsewhere show: That castor oil is highly satisfactory as a purgative after oil of chenopodium, not only by virtue of its purgative properties, but by virtue of a protective action aside from this. It will save the lives of animals receiving lethal doses of oil of chenopodium, when administered with the drug or an hour later, and will save animals given lethal doses of oleoresin of male-fern when given with the drug. Calomel will also save animals when given with lethal doses of male-fern. Liquid petrolatum affords but little protection against lethal doses of chenopodium and diminishes the anthelmintic efficacy. Olive oil retards the passage of chenopodium from the stomach and increases gastric absorption; it does not protect against lethal doses. Some medical men prefer magnesium sulphate to castor oil; our data on this point are inadequate. Magnesium sulphate is not well suited to dog practice.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 239, 1920.

(Reprinted from the *Journal of Industrial and Engineering Chemistry*, Vol. 12, No. 1, page 50, January, 1920.)

PURIFIED CRESOL (CRESYLIC ACID).

By Herbert C. Hamilton.

(Medical Research Laboratories, Parke, Davis & Company, Detroit, Mich.)

One of the minor problems arising from the war because of interference with importations from Europe was that of obtaining a substitute or equivalent for trikresol, a proprietary article imported from Germany and extensively used as a preservative and disinfectant. As has been shown to be true in many other instances, it is equally true in this case that there is no lack in America either of crude material or of ability to purify it. There was required only the incentive.

Trikresol is so named because it is a mixture of the three isomeric cresols naturally occurring in coal tar. These three cresols are identical in composition, but have different physical and bactericidal properties. These differences, however, are unimportant and nothing of practical value results from their separation.

Trikresol, while useful as a general antiseptic and germicide, with a phenol coefficient of 2½ to 3, found its most extensive application as a preservative for serums, vaccines, and similar biologic substances. Careful research has proved that for this purpose, with one exception,* no other antiseptic has been found entirely suitable, either because of its efficiency or the toxic or irritating action when absorbed from a hypodermic injection.

The cresols have practically the same toxicity as pure phenol, as shown in the accompanying table, but the corrosive action is so low and the germicidal value so high in comparison, that the use of phenol as a germicide is no longer logical. To illustrate: cresol with a coefficient of 3, when diluted 1 to 60, is equal in every respect to a 5 per cent solution of phenol, while the toxicity

*Carl Voegtlin, Hygienic Laboratory, *Bulletin* 96.

of the solution is only one-third as great because of the degree of dilution, and the corrosive action, while not measurable with accuracy, is less than one-third as great.

Superficially trikresol is identical with the cresols of coal tar, since an average sample of the latter contains not over 5 per cent of constituents other than the cresols. But it was very promptly observed that cresol, as it appears on the market under various names, is inapplicable for use as a serum preservative because of three specific reasons, all of which are inter-related, namely:

1—Incomplete solubility
2—Disagreeable odor
3—Color

Incomplete solubility is due to the presence of one or more of three substances, naphthalene, colored compounds formed apparently at the expense of the cresols, and phenols of higher boiling point and less solubility than the cresols.

The disagreeable odor is largely due to pyridine and partly to the naphthalene, which have been incompletely separated in preparing the crude phenol.

The origin of the color which appears in cresol and phenol is more or less uncertain. It is probably not always due to the same cause, but may in some cases be due to impurities in the cresol, and in others to incidental conditions, such as the effect of light or air or the action of alkali from the glass container.

It is said that the germicidal value of a highly colored lot is greater than that of a clear straw-colored sample. This, however, is probably a hastily drawn conclusion from insufficient evidence, since different lots of the same color are found to differ much more than a water-white and a colored sample from the same lot. Redistillation corrects the color and can improve the solubility and odor, but not to a sufficient extent.

The development of color appears to be a property not only of cresols but also of pure phenol, and no method has been devised by which such a change can be entirely prevented. The coloring matter appears to be a new constituent and to have properties entirely different from those of the original cresols. It remains behind on redistillation, but further quantities form so that only the freshly distilled material is entirely colorless.

Gibbs[1] ascribes the development of color by the action of sunlight to a labile hydrogen atom and describes experiments with the three cresols in which coloration occurred in varying times with the different ones, but all were affected in the same way.

This, however, does not explain the immediate cause of this coloration. A sample of a freshly redistilled lot was set in the sunlight and another was kept in an amber bottle in the dark. The first, after three months, was very slightly tinged with pink, the other was decidedly reddened. This is not an isolated case but was a careful demonstration of what frequently occurs in practice with large lots. Sufficient observations have not been made to arrive at a theory as to the cause or causes of the change, and no method has consistently prevented its recurrence.

The disagreeable odor is due to pyridine and naphthalene which one would naturally think were eliminated in the process of separating the cresols from the creosote oil. In this process the acid constituents of the oil are combined with an alkali such as caustic soda or lime, and in this form should be readily separated from the neutral and basic substances. In fact only a very small percentage of these bodies remain with the cresols and are dissolved with it when its alkaline combination is broken up, as it is in practice, with carbonic or sulfuric acid. The odor of crude carbolic acid is not distinctly like either impurity, since the natural odor of the cresols masks the others until they are unrecognizable. Distillation carries these impurities over and little improvement can be accomplished by this step as the sole purifying process.

Nevin and Mann[2] depended on separation by redistillation to obtain the proper fraction, but this, as noted above, while removing the colored and some of the insoluble impurities, fails to remove pyridine and naphthalene, which are responsible for the odor. While this odor is perhaps negligible, it is easy to detect the difference between two lots of cresol, one purified to remove the odorous impurities, as will be described later, and the other purified by redistillation only.

In my experiments an attempt was made to study the sodium cresylate compound when prepared in molecular proportions. In concentrated solution no observable separation takes place to in-

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[1] *J. Am. Chem. Soc.*, 34 (1912), 1190.

[2] *J. Am. Chem. Soc.*, 1917, 2752.

dicate that purification by this means is feasible, but on further dilution the naphthalene crystallizes out in a well-recognized form and often in considerable quantities. This can be filtered out, but on again recovering the cresols no material improvement in odor results, because the naphthalene is the less objectionable of the two.

My next experiments were carried out having in mind the examination of sodium cresylate in solid form to see if impurities could be detected, identified and removed by tests applied to the dried or crystallized material.

In the process of evaporating the solution it was observed that the vapors smelled distinctly of pyridine, and further, that after a certain time no odor of this character could be detected. Carrying this experiment to its conclusion and recovering the cresols, they were found to be practically free from the objectionable odor of the crude cresols, and on redistillation a water-white soluble product was obtained with no odor but that of the pure cresols.

The practical working out of this process is as follows: Dissolve the crude cresol in a solution of caustic soda molecularly equivalent, using sufficient water to dilute the sodium cresylate to a 25 per cent solution. Then boil or drive live steam through the solution until the odorous impurities have passed off with the steam. If the solution is boiled over an open flame, care is necessary to avoid concentrating the solution too much as the cresylate breaks up and free cresol is volatilized and may take fire. It is important to add water to replace that lost by evaporation.

The time necessary to vaporize the impurities varies with the amount present, and can be determined by smelling. "The nose knows" when the pyridine is gone. The solution should be allowed to become cold and then observed to see if naphthalene or other neutral oils are present. Any floating oil can be skimmed off, while the naphthalene, if any remains unvolatilized, can be removed by filtration or centrifuging.

Treatment with sulfuric acid equivalent to the alkali originally used will break up the cresylate and set free cresol, which can be recovered as a supernatant layer over the sodium sulfate solution. Separation should be very complete, as the water otherwise present causes trouble in distilling.

The removal of these two impurities, which rarely amount to

more than 5 per cent of the cresols, is therefore equivalent to a complete purification of the substance, since the color is automatically removed by redistillation, and a careful observation of the temperature of distillation at the end of this step insures the removal of the higher boiling phenols, which are less soluble than the cresols and may for that reason be considered as impurities.

Toxicity Assay.

Sample .... Purified cresols
Animal .... Guinea-pigs
Method .... Subcutaneously

Cresol.

Wt. of Animal.	Dose per Kilo.	Result.
0.572	0.6	Recovered
0.611	0.6	Recovered
0.557	0.7	Died
0.640	0.7	Died
0.572	0.8	Died

Phenol.

Wt. of Animal.	Dose per Kilo.	Result.
0.437	0.5	Recovered
0.480	0.5	Recovered
0.446	0.6	Died
0.480	0.6	Died
0.570	0.6	Died
0.340	0.7	Died

Toxicity about 90 per cent of that of phenol: Worth Hale, Hyg. Lab., *Bulletin* 88; James Leake and Hugh B. Corbin, Hygienic Laboratory, *Bulletin* 110.

Germicidal Assay.

Sample ....Purified cresols
Method.....A. P. H. A. phenol coefficient method*
Organism...*B. typhosus*

Dilutions	Time and Results			
Sample	5	10	15	20 minutes
1-300	—	—	—	—
1-350	—	—	—	—
1-400	+	—	—	—
1-450	+	+	—	—
1-500	+	+	+	+
Phenol				
1-100	—	—	—	—
1-110	+	—	—	—
1-120	+	+	—	—
1-130	+	+	+	+
1-140	+	+	+	+

Coefficient 3.6

*Committee Report, *Am. J. Pub. Health,* 8 (1918), 506.

The cost of the process is inconsiderable, since no complicated chemical or mechanical steps are necessary. It is evident from observation of the steps in the process that no unusual equipment is needed and only the commonest chemicals are employed. It is evident, therefore, that here again the German chemists profited at our expense for many years while the crude materials waited only for proper development.

The logical place for the economical production of the refined cresols is where the crude material is first separated from the oils distilled from coal tar. These crude phenols, necessarily dissolved in alkali to separate them from the neutral oils, can, at that point, by suitable means, be freed completely from their impurities, and after fractional removal of the phenol proper, the cresols could then be recovered in pure form with one operation.

The production of purified cresols is, therefore, a logical opening for American enterprise, as well as American resources, for here, as in Europe, are immense supplies of coal tar on which to draw for crude materials.

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 240, 1919.

(Reprinted from the *Bulletin of Pharmacy*, November, 1919.)

THE IDENTITY OF COMMERCIAL BLUE FLAG.

By Oliver Atkins Farwell.

(Department of Botany, Parke, Davis & Co., Detroit.)

The great bulk of commercial blue flag rhizome presents, when fractured, a cortex that has a purplish-brown color. During the past two or three years, an occasional lot has passed under my observation which showed, when the rhizome was fractured, either a very pale purplish-brown or pink to yellowish-white cortex. No other difference was observed, except that the rhizomes appeared to average somewhat smaller.

Steps were taken to have flowering plants with rhizomes attached collected and sent to me for identification. This was accomplished in the case of the pinkish rhizome during the summer of 1919. It proved to be *Iris versicolor* Linné. On those plants with white rhizomes the flowers were too far gone to determine whether they were typical *Iris versicolor* Linné or the variety *Virginica* (Linné) Baker. I have therefore failed, for the time being at least, to correlate the variations in the floral characters with the variations in the color of the rhizomes.

Receipt of entire plants showing the ordinary purple-brown rhizomes immediately confirmed the opinion previously held that it was specifically distinct from the plant producing the lighter-colored and smaller rhizomes. These purple-brown plants, however, had neither flowers nor fruits, but a guess was made that they might prove to be *Iris caroliniana* Watson. Later entire plants with fruits were received and our guess was proved a certainty.

In so far as my experience goes the blue flag of commerce, during the past quarter century, has been chiefly if not wholly the rhizomes of *Iris caroliniana* Watson. The rhizomes in both *Iris versicolor* and *Iris caroliniana* branch in the same manner, from the terminal point of the season's growth, at which point they are

much enlarged. Those of *Iris versicolor* will average up somewhat smaller in size than those of *Iris caroliniana,* which are often 3 centimeters wide at the enlarged joints. The proportion of cortex is about the same in both, one-fourth the diameter of the rhizome.

Microscopically, in transverse section, the only difference noticeable was in the size of the cells, those of *Iris versicolor* being generally smaller than those of *Iris caroliniana;* the parenchyma cells in the latter ranged from .04 to .12 of a millimeter, while those of the former were from one-half to two-thirds as large. The endodermal cells of *Iris caroliniana* varied in outline from oval to circular and from .018 to .067 millimeter in horizontal diameter by .018 to .035 millimeter vertically, with comparatively thin walls of about .0015 millimeter. Those of the other were smaller and had thicker walls and generally were more round in outline.

Under these circumstances it seems to be in order now to amend the definition of *Iris versicolor* of the National Formulary to admit the use of *Iris caroliniana* Watson, as well as the common blue flag, *Iris versicolor.*

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 241, 1920.

(Reprinted from the *Journal of the American Pharmaceutical Association,* Vol. IX, No. 2, February, 1920.)

THE STANDARDIZATION OF BLOOD COAGULANTS.*

By Herbert C. Hamilton.

Hemorrhage, whether due to an abnormal condition of the blood or to lacerated tissues intentional or otherwise, has long been the subject of careful consideration.

Wiggers[1] found that in the different stages of pulmonary hemorrhage pituitary extracts, by their action in raising systemic arterial pressure, while at the same time lowering that in the pulmonary circuit, are peculiarly adapted to its control. This agent, however, has no direct effect on the rate of blood coagulation, its peculiar effectiveness being due to its action on the heart and vessels.

Adrenalin is frequently used and with very good results, since it acts to constrict the vessels locally, retarding the flow of blood and permitting the formation of the clot. But so far as known, it has no action on the character of the blood to hasten coagulation. (Note: See Cannon and Gray, Am. Jour. Phys., vol. 34, p. 232.)

In hemorrhage, while any step which will favorably influence coagulation is to be recommended, no procedure is equal to one which will shorten the coagulation time of the blood itself, promoting the formation of the clot at the exposed surface of the ruptured vessels and thus more quickly sealing them.

The accepted theory of blood coagulation and its remaining fluid in the vascular system is that the substance essential to the formation of fibrin from fibrinogen is thrombin, which is held in the form of prothrombin by a hypothetical antithrombin or antiprothrombin. Possibly both substances are present, the one to hold the prothrombin, the other to destroy or combine with any thrombin which may be formed. It is very difficult seriously to

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*Read before Scientific Section, A. Ph. A., New York meeting, 1919.
[1]Wiggers, *Archives of Int. Med.,* 1914, p. 14.

disturb the equilibrium which maintains intravascular fluidity. When the tissues are lacerated, however, the shed blood, if of normal character, will soon form a clot and tend to seal the wound.

From the edges of the wound the tissue fluids exude, and from the blood platelets, which quickly disintegrate when exposed to the air, similar substances are set free. These two sets of fluids mix with the blood and start the process of coagulation, which consists essentially in liberating the prothrombin from its antithrombin binding and in promoting the reaction first between prothrombin, thrombokinase and calcium ions by which thrombin is produced, then between the thrombin and fibrinogen, by which fibrin is split off and the clot formed. Morawitz[2] designated this substance which initiates coagulation as thrombokinase.

The change which has taken place appears to be twofold. If we consider coagulation as an abnormal condition of the blood, the first departure from the normal is the cessation of metabolism by which the globulins are returned to the blood. When cytoglobulin,[3] the anticoagulative substance—antithrombin—is no longer poured into the blood, prothrombin is set free and predominates.

With the calcium of the blood and in the presence of the tissue fluid containing thrombokinase, which now appears and constitutes the second abnormal condition, thrombin is produced and the clot-formation at once proceeds if the normal blood has been in proper equilibrium. Some have assumed that the office of the thrombokinase is twofold, but proof has been obtained that the latter has no action on the antithrombin.[4]

Antithrombin seems to be a product of the liver, and its activity or the quantity present in the blood may be greatly increased by the injection of peptone, which can render the blood incoagulable.

Thrombokinase, the name originally given to the tissue and cell juices which initiate coagulation, was believed by Wooldridge[5] to owe its effectiveness to the lipoid lecithin, although he also observed that lecithin, derived from the yolk of eggs, is inactive in this respect.

[2]Morawitz, *Deutsch Archiv. f. Klin. Med.*, 179, p. 1.
[3]Schmidt, "Zur Blutlehre.," Leipzig, 1893.
[4]Lapenta, V. A., Paper read before Delaware-Blackford Co. Medical Societies at Muncie, Ind., Apr. 4, 1919.
[5]Wooldridge, "Beitrage zur Physiologie," 1887.

Howell[6] and his co-workers established the fact that the active agent is lipoid cephalin, which exists in largest proportion in the brain cells, but also occurs in most tissues. Howell further states that cephalin, however carefully prepared, has only a limited period of activity and may be dangerous for intravenous administration. Dr. Hess stated that it should never be used intravenously, although intravascular clotting may not be the invariable consequence.

The value of thrombin, prepared from fresh blood serum, is also limited since, unless treated to prevent change, it soon reverts to the inactive metathrombin. In any case the action of thrombin is limited to the amount present since the reaction between thrombin and fibrinogen is strictly quantitative. (Rettger.[7])

Davis[8] found that intravenous injection of purified thrombin in relatively large quantities, instead of causing intravascular clotting, had the effect of prolonging the clotting time. There appeared to be an increased formation of antithrombin to compensate for the excess of thrombin.

Blood serum, containing fresh active thrombin, or serum treated to retain the thrombin in an active form, when used in sufficient quantity is an effective agent in shortening the coagulation time of hemophilic blood.

In the case of hemophiliacs there is an abnormal condition due either to an excessive amount of antithrombin or a deficiency of prothrombin, or in any particular case both conditions may prevail. It is logical, therefore, to keep in mind the conditions possibly present and to use a product capable of meeting all the requirements.

From the time of the earliest attempts to influence the coagulation time, fresh blood and blood serum have been used. But the difficulty and danger in the former, and the unreliability of the latter, stimulated research to develop a more dependable coagulant.

Among those resulting from various researches are products from blood platelets,[9] from brain substance,[10] from blood serum,[11] from tissue extracts and from a combination of these.[12]

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[6]Howell, "The Coagulation of the Blood," 1916-17.
[7]Rettger, *Amer. Jour. Physiol.*, 24, 406, 1909.
[8]Davis, *Ibid.*, 38, 233, 1911.
[9]Fonio, *Corresp.-Blatt f. Schweiz Aertzl.*, No. 13-15, 1913.
[10]Hess, *Jour. A. M. A.*, 1915, p. 64.
[11]Clowes and Busch, *N. Y. Med. Jour.*, 97, 16, 1913.
[12]Lapenta and Walters, *Therapeutic Gazette*, 34, 24, 1918.

Coagulen is a powder prepared from blood platelets by fractional centrifuging followed by careful desiccation and dilution with milk sugar. It is claimed to be a preparation of lipoid material, one gramme of which represents 20 grammes of dried blood. The active agent of coagulen is a thromboplastin which, according to Howell and others, is nothing else than cephalin and identical with the extract of brain substance.

Thromboplastin, kephalin and hemagulen are names applied to the extract of brain tissue. This is obtained either by extracting with Ringer's solution an artificial blood plasma, thus obtaining more or less of a suspension, or by extracting with an ether-acetone-alcohol reagent, by which a yellow lipoid residue is obtained.

Most of these are for local application only or principally, since there is danger of intravascular clotting if intravenously administered.

Blood serum contains no fibrinogen and probably no prothrombin or thrombokinase. It does contain thrombin, although in a more or less inactivated form, due to the gradual recombination with antithrombin and the production of a so-called metathrombin.

Coagulose is a purified product obtained by precipitation of fresh horse serum by which an easily soluble powder is obtained, representing practically the active thrombin. The dose is 10 cc of the solution.

Hemostatic serum is a coagulant in liquid form, of which the dose is only 1 to 2 cc for any form of administration. It contains three distinct substances, all of which are concerned in the process of blood coagulation: 1st, prothrombin, the form in which thrombin exists in the blood; 2nd, thrombokinase or tissue extract, but not derived from brain tissue; and 3rd, a substance which for lack of a more specific name is called anti-antithrombin, a substance similar to antitoxin in that it neutralizes the excess of antithrombin and lowers the anticoagulative power of the blood.

With hemostatic serum, therefore, the surgeon is in a position to counteract abnormal conditions of the blood whichever one is responsible for the slow coagulative power, and at the same time to initiate the process of coagulation when the prothrombin content has been augmented and the antithrombin rendered inactive.

The statement has been made that some of the coagulants men-

tioned have only a limited period of usefulness, unless proper precautions have been taken to remove or inactivate certain accompanying substances. How can it be shown that these precautions have been taken? Clinical reports have shown the effectiveness of freshly prepared extracts of brain tissue or blood platelets. But in the life-or-death cases when a blood coagulant is required are we to depend on the fact that the efficiency of a similar preparation had once been demonstrated?

I have tested three extracts of brain tissue and in only one case has any material shortening of the coagulation time been observed. Only one extract of blood platelets was available for test, and in no case, by any means of testing, was it possible to demonstrate coagulative properties.

It is not possible by any known method of examination other than one closely allied to the physiological to determine whether a coagulant of this character is active.

As in other cases where a valuable medicinal agent is not amenable to chemical standardization, it became necessary to evolve a physiological test in order to have some dependable means of measuring the hemostatic value of these agents. While clinical evidence is, in the final analysis, the sole criterion, clinical evidence is usually slow and often unsatisfactory. As a means of laboratory control over the activity of a product of such vital importance some more flexible method is imperatively demanded.

For testing blood derivatives, such as normal horse serum and coagulose, a method has been applied by which the coagulation time of horse plasma is measured. The test blood is drawn into a solution of sodium citrate or oxalate and the mixture centrifugalized to throw out the corpuscles to facilitate observation. Definite proportions of the coagulant and plasma are mixed and the time recorded when a firm clot is formed. A standard is selected, the maximum period during which the plasma may remain uncoagulated. This test, as shown by the chart, is very satisfactory as a laboratory measurement of value where applicable.

A similar test is theoretically applicable to all the agents mentioned by eliminating the decalcifying agent and keeping the blood plasma fluid by careful drawing and at low temperature.

There is no immediately apparent reason why it is not appli-

cable to hemostatic serum, a product which combines the essential elements of all the other blood coagulants, but repeated experiments have failed to shorten the coagulation time of horse plasma under the conditions described. It was necessary, therefore, to work out a different method since the clinical test—the final deciding criterion—is ordinarily not immediately available. (Note: With certain modifications it is possible to apply this test.—The author.)

The logical method for measuring coagulation time is that for which the Bifi-Brooks coagulometer was devised. A large drop of blood, a series of loops in a platinum wire, and means for keeping the film of blood in the loops moist and warm, are all that is required for carrying out the test. Very accurate measurements can be made on blood from the human subject and on any one with a coagulation time sufficiently long to permit measurement when the time is greatly shortened.

My experience with the dogs' blood, however, seemed to prove that the apparatus is not readily adapted to the test of coagulation time after hemostatic serum had been administered. The fibrin film in the loop forms so promptly that the shortened time cannot be accurately measured. It is obscured by variations in the behavior of the films of fibrin which do not uniformly retain the web across the loop. This limitation was sufficient to prevent that accuracy which is essential to a proper reading of results.

The method that was finally adopted, as a tentative measure at least, seems not to differ materially from that used for coagulose, except that the action of the hemostatic serum is *in vivo* rather than *in vitro*. The method in detail makes use of an anesthetized dog to facilitate making intravenous injections and the frequent withdrawals of blood for observation.

The dog is anesthetized with chloretone (trichloro-tertiary-butyl alcohol) by intraperitoneal injection.[13] A femoral vein and a carotid artery are opened and glass cannulæ inserted, for injecting the solution and drawing out samples of blood. (Note: It is better to plug the canula than to clamp the artery. A convenient amount of blood, about 3 cc, is drawn into a clean test-tube and immediately placed in water at 40° C. At one-minute intervals it is observed to note the beginning and progress of coag-

[13] Rowe, L. W., *Journ. Pharm. and Exp. Therap.*, 9, 107, 1916.

ulation, the amount of coagulation being roughly recorded as— (minus) when no evidence of fibrin formation has appeared, 1 (meaning 1 plus) when this is first observed, 2 (++) and 3 (+++) as it proceeds, and 4 (++++) when coagulation is complete and the tube can be inverted without disturbing the clot. In some cases a tough film forms on the surface while the volume of blood below remains fluid. This should be broken so that correct observations can be made.

Before the second and subsequent samples of blood are drawn, the cannula must be carefully cleaned to remove the clot and all adhering particles, and an amount of blood about equal to the sample to be used is discarded.

Several samples are drawn and tested to determine the normal coagulation time before making the injection. After injection of hemostatic serum the first evidence of shortened coagulation time usually does not appear in less than 15 minutes. It may be delayed as long as 1 hour before any consistent shortening of the time is observed. Usually, however, it is advisable to test a sample of blood every 15 minutes. If no effect from an active coagulant is observed in 1½ hours, the dog is probably highly resistant to the effect of a hemostatic and must be discarded. While such a condition is not easily explainable, failure is not an unusual occurrence in physiological testing.

Another difficulty which occasionally appears in attempting to carry out tests by this method is, that shortening in the coagulation time occurs with no apparent reason. This has also been observed by Drinker and Drinker,[14] but our results differ from their observations because the hemorrhage is not so severe as is shown in their technic.

The standard for hemostatic serum is a shortening of the coagulation time to one-third or one-fourth the normal time for that test animal. Clinical tests have repeatedly verified the correctness of the results by this test and seem to establish definitely the fact that this is a dependable means of assay. The method was suggested by some work described by Howell[15] in which cephalin was injected intravenously. The production of thrombin was demonstrated by this method just as definitely as when added to the blood outside the body.

[14]Drinker and Drinker, *Amer. Jour. of Physiol.*, 38, 233, 1915.
[15]Howell, "The Harvey Lectures," 1916-1917, p. 296.

It is possible and even probable that by using mixtures of pure fibrinogen and thrombin, or other combinations of blood constituents, the test will be further simplified and thus eliminate the test animal; experiments so far have not proved satisfactory.

Until such work has been successfully prosecuted, we are content to depend on the physiological test, which seems in every way to run parallel to clinical results.

PROTOCOLS OF TESTS.

In this and all subsequent tables the first column of figures refers to the time of observation of the sample of blood.

At the head of the columns that follow is the actual time when the sample of blood is drawn or sample of coagulant is injected.

The figures below the time are symbols:

1, signifies beginning of fibrin formation; 2 and 3, progressive clotting; 4, solid clot when tube is inverted.

Coagulation Time Tested by Effect in Vivo.

Hemostatic Serum—℞ 050879.

Tested Aug. 22, 1919.

Minutes	10:00	10:10	10:20	10:22	10:51	11:01	11:08	11:11	11:18	11:36
1	—	—	—	Inj.	—	1	3	2	3	4
2	—	—	—	2	—	2	4	3	4	
3	1	1	—	Cc.	1	2		4		
4	1	2	1	sample	1	3				
5	2	2	2		2	4				
6	2	3	2		3					
7	3	3	3		4					
8	3	3	3							
9	4	4	4							

Coagulation time shortened from 9 minutes to 1 minute.

Sample A.—From Blood Serum.

Tested July 22, 1919.

Minutes	1:13	1:57	1:58	2:16	2:35	3:40
1	—	Inj.	—	—	—	—
2	—	solution	—	1	1	—
3	—		—	2	2	—
4	—		1	3	4	—
5	1		2	3		—
6	2		3	4		1
7	2		4			2
8	3					3
9	4					4

Coagulation time shortened to one-half normal.

Sample A.—From Blood Serum—Coagulation Test.

Using Decalcified Sheep Plasma.

Minutes	Normal	0.025	0.05	0.1	0.2	0.4	0.6
15		1	1	1	2	4	4
25		1	1	1	2	4	4

Coagulation complete in 15 minutes with 0.4 Gm. of Sample A.

In the serum tests the columns are headed by the amount of sample added to plasma.

Sample B.—Tests of Action in Vivo.

Tested July 23, 1919.—From Blood Platelets.

Minutes	10:27	10:33	10:40	10:53	10:55	11:01	11:11	11:18	11:21	11:38
1	1	1	1	Inj.	1	1	1	Inj.	1	1
2	2	1	2	2 Cc.	3	2	2	2 Cc.	2	2
3	3	2	3	sample	3	3	3	more	3	3
4	3	3	3		4	3	3	of	4	4
5	4	3	4			4	3	same		
6		4					4			

Same Test Continued.

Minutes	1:12	1:20	1:31	1:40	1:53	2:17	3:27
1	1	Inj.	1	2	2	1	1
2	1	2 Cc.	2	3	3	2	1
3	2	more	3	4	3	3	2
4	3	of	4		4	4	3
5	4	same					3
6							4

Sample B.—(Second Test).

Minutes	9:58	10:05	10:15	10:21	10:29	10:40	10:53	11:02	11:15	11:39	11:46	1:14
1	—	—	1	—	Inj.	—	—	—	—	1	1	1
2	1	1	2	1	4 Cc.	—	—	—	—	2	2	1
3	1	1	2	1		1	1	1	1	3	2	2
4	2	2	3	2		3	1	1	2	3	3	2
5	3	2	3	3		4	2	2	3	3	4	3
6	3	3	4	4			3	3	4	4		3
7	4	4					4	4				4

Coagulation time not materially shortened.

Sample B.—From Blood Platelets.

Coagulation tests using horse plasma *in vitro.*

Minutes	Normal	0.05	0.1	0.12	0.15
1	—	—	—	—	—
2	—	—	—	—	—
3	—	—	—	—	—
4	—	—	—	—	—
5	—	—	—	—	—
6	—	—	1	—	1
7	—	—	1	1	1
8	1	1	1	1	1
9	1	1	1	1	1
10	1	1	2	1	2
11	1	1	3	1	2
12	2	2	4	2	2
13	3	3		3	3
14	4	3		4	4
15		4			

Practically no shortening of the coagulation time resulted from use of this agent.

Sample C.—From Brain Tissue.

Tested July 23, 1919, by action *in vivo.*

Minutes	10:13	10:22	10:31	10:34	10:47	11:00	11:15	11:29	11:53	1:17
1	—	—	—	Inj.	—	—	—	—	—	—
2	—	—	—	2 Cc.	—	—	—	—	1	1
3	1	1	1	sample	1	1	1	1	2	1
4	1	1	2		1	1	1	2	3	2
5	2	2	3		2	2	2	4	3	2
6	2	3	3		2	3	3		3	3
7	3	4	4		3	4	4		4	3
8	4				4					4

Test continued.

Minutes	1:28	1:33	1:38	1:50	1:55	2:34	2:58	3:12	4:11	4:21
1	Inj.	1	1	1	1	3	1	3	3	2
2	4 Cc.	3	2	2	2	3	2	4	4	3
3	sam-	4	2	3	3	4	3			4
4	ple		4	4	4		4			

The action was both prompt and lasting when a second injection of 4 cc was made. Not only did it appear in 5 minutes but the shortened coagulation time extended over a period of 3 hours.

The dog was alive the next morning, although useless for further examination. The effect of the thromboplastin was so strong as to clot the blood in the artery making it difficult to obtain samples.

Shortened coagulation time from 7 minutes (original) to 2 minutes.

Sample C.—From Brain Tissue.

Coagulation test using horse serum.

Minutes	Normal	0.05	0.1	0.15	0.2
1	—	—	—	—	—
2	—	—	—	—	—
3	—	—	—	—	—
4	—	1	1	—	1
5	—	2	2	—	2
6	—	4	4	1	4
7	—			4	
8	—				
9	—				
10	1				
11	3				
12	4				

Coagulation time shortened to 6 minutes.

Sample D.—From Brain Tissue.

Material emulsified with normal salt solution.—Tested July 23, 1919.

Minutes	2:30	3:30	3:40	3.41	3:54	4:05	4:22
1	—	—	Inj.	—	—	—	—
2	—	—	2 Cc.	1	1	1	1
3	1	1	emul-	1	1	1	1
4	2	1	sion	2	1	2	2
5	2	1		2	2	3	3
6	3	2		3	2	4	4
7	3	3		3	3		
8	4	4		4	4		

Sample D (Second Test).

Tested Aug. 11, 1919.

Minutes	9:58	10:06	10:50	10:53	10:57	11:11	11:21	11:31	11:33
1	—	—	—	Inj.	—	—	—	—	—
2	1	1	1	2 Cc.	1	1	1	1	1
3	2	2	2		2	2	2	2	2
4	2	3	2		2	3	3	3	3
5	3	3	3		3	4	3	3	4
6	3	4	3		4		4	4	
7	4		4						

Coagulation time scarcely affected.

TEST OF HEMOSTATIC SERUM—℞ 049606.

Same day. Same dog.

Minutes	1:15	1:26	1:28	2:17	2:30	2:45	2:55
1	—	Inj.	1	—	—	1	2
2	1	2 Cc.	2	1	1	2	4
3	2	Hem.	3	2	2	4	
4	2	Serum	3	3	3		
5	3		3	4	4		
6	4		4				

Coagulation time shortened from 6 to 2 minutes.

Sample E.—From Brain Tissue.

The material was injected in form of an emulsion with normal salt solution.—Tested Aug. 9, 1919.

Minutes	10:49	10:56	11:13	11:25	11:40	1:45	2:25
1	—	—	Inj.	1	1	1	—
2	1	1	5 Cc.	1	2	2	1
3	2	2	emulsion	2	3	3	2
4	2	2		3	4	3	2
5	3	3		4		4	3
6	3	3					4
7	4	4					

Coagulation time only slightly affected.

TEST OF HEMOSTATIC SERUM.

Same day. Same dog.

Minutes	2:25	2:35	2:37	3:10	4:09	4:15
1	—	Inj.	1	1	2	2
2	1	2 Cc.	2	2	2	4
3	2	of	3	3	4	
4	2	sample	3	4		
5	3		4			
6	4					

Coagulation time shortened from 6 minutes to 2 minutes in 1⅔ hours.

An illustration of one form of failure which occasionally occurs in attempting to test a coagulating agent.

May 21, 1919.

Minutes	10:33	10:43	10:49	10:52	11:48	1:31	2:11	3:00
1	—	1	1	4	2	1	1	2
2	—	2	4		4	3	2	3
3	1	3					3	
4	2	4						
5	2							
6	2							
7	3							
8	3							
9	4							

No injection was made into this dog as the coagulation time would not remain uniform at any time.

ABSTRACT OF DISCUSSION.

DR. PITTENGER: I just wish to state that during the past several months I have had considerable experience in testing blood coagulants. Although we have not done as much work towards perfecting the method as Dr. Hamilton brings out in his paper, we have obtained practically the same results, *viz.*, that there are quite a few preparations on the market which appear to

be valueless as to their coagulant properties, and some others which apparently do the work very well.

I employed a method somewhat similar to his in which I used very small paraffined glass test-tubes. The only difference between Dr. Hamilton's technique and that which I employed was that our experiments were carried out at room temperature. We anesthetized a dog, tied a paraffin-coated cannula into the carotid artery, and then withdrew four or five tubes of normal blood. The coagulant was then injected into the saphenous vein and samples of blood drawn into paraffined tubes every two minutes. The clotting time of the normal blood was then compared with the clotting time of the samples taken after the coagulant was injected. We found that normal blood clotted in about fifteen to twenty minutes, while after an effective coagulant was injected the clotting time was reduced to four to eight minutes. We found, however, that a few of the preparations on the market instead of increasing the coagulability of the blood actually retarded coagulation. In one case after injecting a so-called coagulant, clotting did not take place in two hours, whereas the normal blood from the same animal clotted in eighteen minutes.

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 242, 1920.

(Reprint from *The Journal of Laboratory and Clinical Medicine,* Vol. V, No. 5, (February, 1920.)

BACILLUS BRONCHISEPTICUS AS THE CAUSE OF AN INFECTIOUS RESPIRATORY DISEASE OF THE WHITE RAT.

By H. Preston Hoskins, V.M.D., and Alice L. Stout, A.B., Detroit, Mich.

(From the Medical Research Laboratories, Parke, Davis & Co., Detroit, Michigan.)

The importance of Bacillus bronchisepticus as a pathogenic microörganism has been firmly established by the investigations of numerous workers, both in this country and abroad. A search of the literature dealing with the bacteriology of various diseases of the smaller animals, for the past thirty years, leaves no doubt that B. bronchisepticus was frequently encountered by the investigators who studied these diseases. In the majority of the reports the completeness of the data does not permit a positive statement, now, to the effect that this or that organism was the same as the one we now call B. bronchisepticus, but the fragmentary evidence dealing with the morphology and cultural characteristics, together with certain statements as to the symptoms shown by the infected animals, really leaves little room for doubt as to the identity of some of these organisms with B. bronchisepticus, especially in the light of our present knowledge regarding it and its pathogenic powers.

BRONCHISEPTICUS INFECTIONS OF OTHER ANIMALS.

It remained for Ferry[1] to publish the first complete description of the organism, to which he first gave the name B. bronchicanis, having isolated it from dogs suffering with distemper. In pursuing his investigations further, Ferry found that the organism did not confine its pathogenic activities to the dog, but that it was associated with distemper-like diseases of other small animals. This caused Ferry[2] to rename the organism B. bronchisepticus. This work was confirmed by the publications of

M'Gowan[3] and Torrey and Rahe,[4] which followed shortly after Ferry published his findings. Up to this time B. bronchisepticus has been reported as having been isolated from dogs, cats, rabbits, guinea pigs, ferrets, monkeys and man, but not from rats. M'Gowan[3] made bacteriologic examinations of rats in his laboratory, while studying infections among his laboratory animals, but did not find B. bronchisepticus in cultures made from the lungs, trachea, back of the nose and heart blood.

It is the purpose of this report to record the finding of B. bronchisepticus in pure culture as the apparent cause of a serious distemper-like disease among white rats. The white rat (Mus Norwegicus albinus) is now being extensively used in certain laboratory investigations, especially in work with glandular substances, vitamins and other accessory food substances, in the study of dietary deficiency diseases. In many laboratories the rats are kept in the same buildings with other small animals, such as guinea pigs and rabbits, or even in close proximity to dog kennels, thereby affording favorable conditions for the spread of such an infection as B. bronchisepticus, probably the most common pathogenic organism found among laboratory animals.

NATURE OF THE DISEASE.

The symptoms shown by affected rats are rather constant. These include loss of appetite, dulness, loss in weight, and respiratory disturbances. The latter are quite characteristic and sometimes are present without the other symptoms mentioned. There is a nasal discharge, which varies in consistency, color and amount, in different cases. Accompanying this are frequent paroxysms of sneezing and a peculiar "rattling" breathing sound. For this symptom the name "snuffles" has been casually applied by laboratory attendants, probably on account of the similarity of the rat disease with the well-known "snuffles" of rabbits. Diarrhea was not noted in any of the cases observed.

The disease may run a protracted course or end suddenly. Few recoveries have been noted. Usually a pneumonia has been found in those cases terminating fatally. No attempt has been made to treat cases of the disease for obvious reasons. It has been the part of economy to destroy all rats showing symptoms and start over again with new stock, placed in thoroughly cleaned

and disinfected quarters. There have been times when it was very difficult to obtain healthy stock from commercial rat-breeding establishments. Shipments have been made of apparently healthy stock, only to have them arrive with some of the rats showing symptoms.

BACTERIOLOGIC EXAMINATIONS.

Material for study consisted of sick rats in various stages of the disease and rats dead of the infection. Cultures were made by plating material from the upper respiratory tract, trachea, lungs, and heart blood. Table I shows that B. bronchisepticus was isolated from two rats dead of the infection, and from eleven others killed for autopsy and cultural purposes. The organism was isolated from the nostrils, nasal sinus, trachea, lungs, and heart blood. Usually the cultures from the trachea were pure, as well as those from the lungs and heart blood in the two cases where the organism was recovered from these sources. Cultures made from the upper respiratory tract, nostrils and nasal sinuses frequently yielded B. bronchisepticus, as well as other organisms, such as staphylococci and pyocyaneus. Gas producers were not found.

At the same time that rats Nos. 20-29 were killed, samples of blood were taken, the serum removed and subsequently used for a series of agglutination tests against B. bronchisepticus isolated

TABLE I.

DATA ON RATS FROM WHICH B. BRONCHISEPTICUS WAS ISOLATED.

RAT NO.	KILLED OR DIED	SYMPTOMS	ORGANISM
2	K	Sick; "rattling" breathing	Sinus
3	K	Sick; "rattling" breathing	Sinus
4	K	Sick; "rattling" breathing	Sinus
7	K	Sneezing, "rattling" breathing	Trachea
14	K	Sneezing	Nostril
19	D	Not observed	Heart Blood*
21	K	Sneezing	Trachea*
24	K	Sneezing	Trachea*
25	K	Sneezing	Trachea*
26	K	Sneezing	Trachea*
28	K	Sneezing	Sinus
29	K	Sneezing	Sinus
31	D	Not observed	Lung*

*B. bronchisepticus in pure culture.

from rats in the present outbreak, as well as cultures of the organism obtained from dogs. (See Table II.)

TABLE II.

AGGLUTINATION TESTS.

(1) B. bronchisepticus (rat) suspensions against homologous rat sera.
(2) B. bronchisepticus (rat)* suspensions against rat sera.
(3) B. bronchisepticus (dog) suspensions against rat sera.
*(Strain obtained from Rat 31)

(1) Suspension of B. bronchisepticus against the serum of the rat from which the strain was isolated.

DILUTIONS	RAT 21	RAT 25	RAT 28
1-8	++	+++	+++
1-16	+	++	+++
1-32	—	++	+++
1-64	—	++	++
1-28	—	++	+
1-256	—	+	+
1-512	—	+	—
1-1024	—	—	—
Controls	—	—	—

(2) Suspension of B. bronchisepticus (Rat 31) against sera of rats sick with disease.

DILUTIONS	RAT 21	RAT 22	RAT 23	RAT 24	RAT 25	RAT 26	RAT 27	RAT 28	RAT 29
1-8	+++	+++	+++++	+	+++	+++	+++	+++	+++
1-16	+++	+++	+++	+++	+++	+++	+++	+++	+++
1-32	++	+++	+++	+++	+++	+++	+++	+++	+++
1-64	++	++	++	++	++	++	++	++	++
1-128	++	++	++	++	++	++	++	+	++
1-256	+	++	++	+	+	+	+	+	+
1-512	–	+	+	–	–	–	–	–	+
1-1024	–	–	–	–	–	–	–	–	–
Controls	–	–	–	–	–	–	–	–	–

(3) Suspension of B. bronchisepticus (Canine Strain) against sera of rats sick with disease.

DILUTIONS	RAT 20	RAT 21	RAT 22	RAT 23	RAT 24	RAT 25	RAT 26	RAT 27	RAT 28	RAT 29
1-8	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
1-16	– – +	+++	+++	+++	+++	+++	+++	+++	+++	+++
1-32	+++	+++	++	+++	+++	++	+++	+++	+++	+++
1-64	++	++	++	+++	++	++	++	++	++	+++
1-128	– –	++	++	++	++	+	++	++	++	++
1-256	++	+	+	++	+	+	+	++	+	++
1-512	++	+	+	–	+	+	–	++	–	+
1-1024	++	–	–	–	–	–	–	–	–	–
Controls	–	–	–	–	–	–	–	–	–	–

In the isolation and identification of B. bronchisepticus, suspected colonies were picked from the agar plates and transferred to various media. All organisms which were ultimately designated B. bronchisepticus failed to produce gas or acid in fermentation tubes containing dextrose, lactose, and saccharose bouillon. The closed arm remained clear with turbidity in the open arm. Litmus milk was permanently alkalinized in 48 to 72 hours, and the growth on potato was yellowish brown (tan). All organisms were actively and progressively motile, Gram-negative, non-spore-forming and failed to produce indol.

DISCUSSION.

It will be noted that with two exceptions the agglutination titers were fairly uniform. One exception, where the serum of Rat 21 was run against the homologous strain of B. bronchisepticus, the suspension did not show any agglutination above a dilution of 1 to 16. The same serum agglutinated a strain of the organism from another rat at a dilution of 1 to 256. The organism from Rat 21 was typical of B. bronchisepticus in all respects. The other exception was in the case of the serum from Rat 20, which strongly agglutinated a canine strain of the organism at a dilution of 1 to 1024. At autopsy this rat showed pneumonic areas in the lungs. We were unable to determine the agglutination titer of the serum of normal rats. All rats on hand had been exposed to the disease and efforts to locate healthy stock were unsuccessful. Serum from normal rabbits does not usually agglutinate B. bronchisepticus above a dilution of 1 to 10.

SUMMARY.

1. Bacillus bronchisepticus has been isolated from nostrils, nasal sinuses, trachea, lungs and heart blood of white rats affected with a serious disease of a distemper-like character.

2. The organism was recovered in pure culture in about one-half of the cases. Other organisms were found with B. bronchisepticus, in the nostrils and nasal sinuses, and once in the trachea.

3. Agglutination tests pointed to the identity of the rat organism and B. bronchisepticus from a canine source.

4. The serum of rats affected with the disease agglutinated both homologous as well as heterologous strains of B. bronchisepticus in comparatively high dilutions. One rat serum showed strong agglutination at a dilution of 1 to 1024.

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(Reprinted from *The Journal of Laboratory and Clinical Medicine*, Vol. V, No. 5, February, 1920.)

BACTERIOLOGY AND CONTROL OF CONTAGIOUS NASAL CATARRH (SNUFFLES) OF RABBITS.*

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The condition among rabbits commonly referred to as "snuffles" has been, more or less, a disturbing element to laboratory workers ever since the animal in question was first used so generally for experimental purposes. It has also of late bid fair to become of considerable economic importance, as the practice of breeding and raising rabbits by many individuals in this country as a means of augmenting the family exchequer has recently developed into an industry of extensive proportions.

On account of the general distribution of this industry and the prevailing custom of exhibiting pure-bred rabbits at the meetings of the various State and local rabbit associations, together with the opportunity offered for the spread of the infection in pet shops and bird stores, the disease has necessarily become rapidly and widely disseminated. This has resulted in an increasing demand for a more comprehensive bacteriologic study of the infectious process, as it now exists, and the development, if possible, of a means of protection against it.

PREVIOUS BACTERIOLOGIC FINDINGS.

One of the earliest descriptions of an epizootic, of bacterial origin, of the respiratory tract of rabbits was given by

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*Presented at the meeting of the Society of American Bacteriologists, Boston, Mass., Dec. 29-31, 1919

Beck[1] in 1893. He described an infectious rabbit disease accompanied by a catarrhal pneumonia and often a fibrinous pleuritis caused by a small bacillus which was aerobic, non-motile, negative to Gram, and which could not be grown on potato, although it thrived on other alkaline media. This bacillus was found to kill rabbits within five days after intrapulmonary inoculations. It took eight or ten days, however, to kill after inoculations directly into the blood or on the uninjured nasal mucous membranes. The organism proved pathogenic for mice and guinea-pigs.

In 1897 Kraus[2] described an epizootic in rabbits which resulted in pneumonia with purulent pleuritis and pericarditis, accompanied by a purulent nasal catarrh and an inflammation of the antrum of Highmore. The infection was produced by a small, Gram-negative, aerobic, motile bacillus which developed a yellowish-brown growth on potato.

Volk[3] in 1902 described a contagious disease of rabbits which always appeared to attack the pleura, pericardium, and endocardium. As the cause of the infection Volk reported a small, aerobic, non-motile bacillus, negative to Gram, which was able to grow luxuriantly on potato. This organism appeared to be extremely virulent, killing rabbits within 36-48 hours with a dose of 0.000001 c.c. of a 24-hour bouillon culture after an intraperitoneal or intrapleural inoculation. This amount inoculated into the uninjured nasal membranes took three days to kill, while 0.005 c.c. injected into the trachea resulted in death within ten days.

Südmersen in 1905[4] described an infectious rabbit pneumonia in which a fibrinous pleuritis and catarrhal lung inflammation appeared. The cause of the disease was a short, thin, occasionally double, actively motile bacillus which was Gram-negative and easily stainable with the ordinary aqueous aniline dyes. On potato it formed a yellowish-brown, wax-like growth and produced gas and indol.

In 1910 and 1911 one of us (N. S. F.)[5, 6] described an organism as causing distemper in dogs, and later, 1912[7, 8] and 1914,[9] as producing snuffles in rabbits and a disease similar to distemper in other small animals. This organism, which the above author ultimately named Bacillus bronchisepticus,

is recognized by the following characteristics: It is a short, slender bacillus, usually found single, but often in pairs. In liquid media it may be seen in long chains or filaments, and, when cultivated directly from the animal body, may be larger and more oval in form. It does not stain by Gram but stains with most aniline dyes and with Löffler's methylene blue often gives a typical bipolar appearance. The organism is actively and progressively motile. It gives a filiform translucent growth on solid media. On potato and Koch's serum it produces a tan-colored moist growth. It does not liquefy gelatin, and does not produce acid or gas in sugar. It gives an alkaline reaction to litmus milk. In broth it produces a persistent clouding with a rather viscid sediment. Indol is negative.

The disease as it was found in rabbits was briefly described by Ferry[9] as follows: "Loss of appetite and flesh with decreased activity are usually the initial symptoms. Diarrhea is an invariable symptom, while a discharge from the nose and eyes (purulent very early) is recognized in most cases; wherein it differs from the guinea-pig. Death in the majority of cases is found in from two to ten days, although the disease is not as fatal as with the guinea-pig. Incubation period from five to seven days. In the early stages B. bronchisepticus is usually found in the respiratory tract in pure culture. It may also be found in the blood and abdominal organs. In later stages of the disease this microörganism is associated with pyogenic organisms of secondary infections."

Ferry also mentioned an uncommonly acute infection which was present among his rabbits, at the same time, due to a bacillus of the hemorrhagic septicemia type. This disease was of extremely short duration and invariably fatal. "Often a slight discharge was observed at the nostrils, but this was not so profuse nor so purulent as in snuffles due to B. bronchisepticus. At autopsy a general invasion of the body with the specific microörganism was the rule." An observation was made at that time which appears to have been substantiated by later facts that "slight discharge (of the nostrils) seems to be a common symptom of most infections in the rabbit."

In 1911 M'Gowan[10] described an organism found in an

epidemic among laboratory animals, including the rabbit (presenting the symptoms of the disease called distemper), which proved identical to the organism just preceding (B. bronchisepticus). The condition among M'Gowan's rabbits was, no doubt, typical snuffles.

In 1913 and 1917 Davis[11, 12] described a Gram-negative, non-motile, polar-staining minute bacillus which was found in subcutaneous abscesses and in snuffles in rabbits.

PRESENT INVESTIGATIONS.

Culture material was obtained from rabbits of various ages and during all stages of the disease, particular attention being paid, wherever possible, to rabbits showing early symptoms of the disease. All cultures were obtained by streaking the material on plates or planting in broth. Previous work, as well as preliminary experiments during the present investigations, showed that the predominating and most consistent colonies were small, round, grayish, translucent, and small, round, white, opaque. These two types alone were employed for isolation work. Smears from other colonies were studied, however, but the findings proved conclusively that they had nothing in common with the primary infectious process.

During the life of the rabbits, cultures were obtained from the nares by passing sterile swabs as far into the nasal cavity as possible. At autopsy cultures were obtained from the nasal sinuses, trachea, heart-blood and various organs of the body.

Access was afforded the investigators for a thorough study of this disease as it presented itself in rabbitries in various parts of the country and under conditions ideal for such a line of work, so that the conclusions are in no way influenced by conditions prevailing in any one locality. Entire rabbitries were placed at the disposal of the investigators for bacteriologic observations as well as for further experimental work, and as a result a large number of animals were studied either experimentally or clinically. With these rabbits, also, were included many which were under observation for the effect of specific bacterial vaccine treatment, both prophylactic and therapeutic.

RABBITS STUDIED BACTERIOLOGICALLY.

1. Private stock (6-months-old doe, Flemish Giant). June 16, 1919. Symptoms 7 months. Thick discharge from nose; sneezing. Cultures from nares. Numerous colonies of a small bacillus (B. bronchisepticus) and a few colonies of Staph. albus.

2. Private stock (young buck, Flemish Giant). June 16, 1919. Sick four weeks. Thick discharge from nose; sneezing. Cultures from nares. Numerous colonies of a small bacillus; B. bronchisepticus in large numbers; few Bact. lepisepticum and few Staph. albus.

3. Private stock (young doe, Flemish Giant). June 16, 1919. Just showing signs of illness; slight watery discharge from nose. Cultures from nares. Small bacillus in large numbers; plate contaminated; B. coli only organism isolated.

4. Private stock (young doe, Flemish Giant). June 16, 1919. Sneezing 3 to 4 days. Cultures from nares; small bacillus in large numbers which proved to be B. bronchisepticus; Staph. albus also isolated.

5. Private stock (buck, Flemish Giant). June 19, 1919. Sick six months; thick discharge. Cultures from nares. Several organisms present; gas-producing organisms of the colon type isolated.

6. Private stock (young doe, Flemish Giant). June 19, 1919. Sick six weeks. Thick, white discharge. Cultures from nares. Numerous colonies of small bacillus; B. bronchisepticus in pure culture.

7. Private stock (young suckling, Flemish Giant). June 19, 1919. Nursing sick mother; sneezing. Killed and posted. Cultures from nares, blood, trachea and nasal sinuses. Numerous colonies in large numbers; B. bronchisepticus in nares and trachea in pure culture; Bact. lepisepticum in sinus.

10. Laboratory rabbit. June 18, 1919. Length of illness not known; thick purulent discharge. Killed and posted. Cultures from nares, trachea and blood. Several unidentified organisms in nares only.

11. Laboratory rabbit. June 18, 1919. History not known; thick purulent discharge. Killed and posted. Cultures from nares, trachea and blood. Bact. lepisepticum in blood; unidentified organisms from nares.

12. Laboratory rabbit. June 18, 1919. History not known; thick discharge. Killed and posted. Cultures from nares, trachea and blood. Bact. lepisepticum in blood; unidentified organisms from nares.

13. Laboratory rabbit. June 18, 1919. History unknown; watery discharge. Killed and posted. Cultures from nares, trachea and blood. Unidentified organisms from nares only.

14. Laboratory rabbit. June 18, 1919. History unknown; thick purulent discharge. Killed and posted. Cultures from nares, trachea and blood. Unidentified organisms from nares only.

15. Laboratory rabbit. June 18, 1919. History unknown; thin watery discharge. Killed and posted. Cultures from nares, trachea and blood. Unidentified organisms from nares; Bact. lepisepticum from blood.

17. Laboratory rabbit. June 19, 1919. History unknown; watery discharge. Killed and posted. Cultures from nares, trachea, sinus and blood. Bact. lepisepticum and B. coli from nares; B. bronchisepticus and B. coli from trachea.

18. Laboratory rabbit. June 19, 1919. History unknown; slight discharge. Killed and posted. Cultures from nares, trachea, sinus and blood. Unidentified organisms from nares; Bact. lepisepticum from sinus.

19. Laboratory rabbit. June 20, 1919. History unknown; watery discharge. Killed and posted. Cultures from nares, trachea, sinus and blood. Bact. lepisepticum from sinus.

20. Laboratory rabbit. June 20, 1919. History unknown; slight discharge. Killed and posted. Cultures from nares, trachea, sinus and blood.

Unidentified organisms from nares; Bact. lepisepticum from trachea and sinus.

21. Laboratory rabbit. June 18, 1919. History unknown. Thick purulent discharge. Cultures from nares. Organisms unidentified.

22. Laboratory rabbit. June 18, 1919. History unknown; thin watery discharge. Cultures taken from nares. Organism unidentified.

23. Laboratory rabbit. June 30, 1919. History unknown; slight watery discharge. Killed and posted. Cultures from nares, trachea, sinus, blood, spleen and kidney. B. bronchisepticus in large numbers in nares and also in pure culture in the trachea and sinus.

24. Laboratory rabbit. June 30, 1919. History unknown; slight discharge. Killed and posted. Cultures from nares, trachea, sinus, blood, spleen, liver and kidney. B. bronchisepticus from trachea and sinus in pure cultures.

25. Laboratory rabbit. June 30, 1919. History unknown; sneezing; no discharge. Killed and posted. Cultures from nares, trachea, sinus and blood. Unidentified organism from nares.

31. Private stock (2-months-old doe, Flemish Giant). Aug. 11, 1919. Ill four days; dead six hours; slight watery discharge. Posted. Cultures from nares and sinus. B. bronchisepticus in pure culture from sinus; B. bronchisepticus with other organism from nares.

32. Private stock (young doe, Flemish Giant). Aug. 11, 1919. Sick few days; slight watery discharge and sneezing. Killed and posted. Cultures from nares and sinus. B. bronchisepticus in pure culture from both situations.

33. Private stock (young doe Flemish Giant). Aug. 11, 1919. Sick five days; slight watery discharge. Killed and posted. Cultures from nares and sinus. B. bronchisepticus in pure culture from sinus.

34. Private stock (buck, fourteen months old, Flemish Giant). Aug. 12, 1919. Sick six months; thick white discharge. Cultures from nares. Staph. albus; B. coli and other gas producers.

35. Private stock (young doe, common rabbit, wet nurse). Aug. 12, 1919. Sick few days; slight watery discharge. Killed and posted. Cultures taken from sinus. B. bronchisepticus in pure culture.

36. Private stock (young wet nurse, common rabbit). Aug. 12, 1919. Sick one week; thick discharge. Killed and posted. Cultures from nares and sinus. B. bronchisepticus from sinus in pure culture; B. bronchisepticus associated with other organisms from nares.

37. Private stock (11-months-old doe, Rufus Red). Aug. 12, 1919. Sick three weeks; thick white discharge. Killed and posted. Cultures from nares and sinus. B. bronchisepticus in pure culture from both places.

43. Private stock (young doe, New Zealand Red). Aug. 13, 1919. Symptoms one week; slight watery discharge. Killed and posted. Cultures from sinus. B. bronchisepticus in pure culture.

44. Private stock (young doe, New Zealand Red). Aug. 13, 1919. Sick few days; slight discharge. Killed and posted. Cultures from sinus. B. bronchisepticus.

46. Private stock (doe, with young, New Zealand Red). Aug. 13, 1919. Sick few days. Young rabbits perfectly healthy. Cultures from nares. Staph. albus.

50. Private stock (young rabbit, Flemish Giant). Well when brought in Aug. 13, 1919; rubbing nose, Aug. 16, 1919; dead, Aug. 18, 1919. Cultures from sinus. Unidentified organism.

51. Private stock (young rabbit, Flemish Giant). Sick when brought in Aug. 13, 1919; discharge from nose, Aug. 15, 1919; sneezing and very sick, Aug. 16, 1919. Killed and posted, August 18, 1919. Cultures from sinus and blood. Bact. lepisepticum in sinus.

52. Private stock (young rabbit, Flemish Giant). Slight symptoms when brought in Aug. 13, 1919; considerable discharge, Aug. 15, 1919; very sick, Aug. 16, 1919. Killed and posted, Aug. 18, 1919. Cultures from sinus and blood. Bact. lepisepticum in sinus.

53. Private stock (young rabbit, Flemish Giant). Well when first seen, Aug. 13, 1919; no symptoms, Aug. 16, 1919; killed and posted, Aug. 18, 1919. Cultures from sinus and blood. Bact. lepisepticum in sinus.

54. Private stock (young rabbit, Flemish Giant). Slight symptoms, Aug. 13, 1919; rubbing nose, Aug. 16, 1919. Killed and posted, Aug. 18, 1919. Cultures from sinus. Staph. albus.

55. Private stock (young rabbit, Flemish Giant). Well when first seen, Aug. 13, 1919; slight symptoms, Aug. 15, 1919; nose moist, Aug. 16, 1919. Killed and posted, Aug. 18, 1919. Cultures from sinus and blood. Unidentified.

60. Private stock (Flemish Giant). Chronic case; thick discharge. Cultures from nares. Gas producer and Staph. aureus.

61. Private stock (Flemish Giant). Chronic case; thick purulent discharge. Cultures from nares. Gas producer and B. bronchisepticus.

Total rabbits examined—40.

CONTROL OF THE DISEASE.

If the control of snuffles is to be attempted it must be handled as any contagious disease of bacterial origin; namely, by the observance of the ordinary sanitary precautions and hygienic measures and the use of specific therapy wherever applicable.

In 1914 one of us (N. S. F.)[8] reported the control of a severe local outbreak of an infection with B. bronchisepticus in rabbits by means of prophylactic inoculations with a specific vaccine containing 100 million bacteria per cubic centimeter. Each animal received the vaccine every third day, starting with one cubic centimeter and doubling the dose at each subsequent injection until three injections had been given. At the same time the author reported the unsuccessful attempt to control an infection due to a bacillus of the hemorrhagic septicemia type; later experiments, however, have taught us that the dose was probably much too small. The most recent work of the authors has shown that a larger dose of both organisms may be given with more satisfactory results; in fact, they recommend the use of a vaccine containing 400 million each of B. bronchisepticus and Bact. lepisepticum and 200 Staph. albus as the initial dose.

According to reports from various localities relative to the use of this vaccine it would appear that about 90 per cent of the rabbits were protected after prophylactic inoculation,

and of those cases where treatment was instituted after the disease was well established, about 50 per cent were relieved of symptoms.

DISCUSSION.

From the results of the present as well as former investigations, it is very evident that snuffles in rabbits is not a single entity, due to any one microörganism, but is more or less of a symptom following an infection of the upper respiratory tract with any one or more of several organisms. To further substantiate this statement, in addition to the findings of the authors already quoted, Ward[13] experimentally produced snuffles in rabbits by the intravenous injections of B. ozenæ, B. proteus and B. bronchisepticus.

It seems to be evident that the ordinary form of snuffles as encountered in the various rabbitries in this country, characterized by a variable nasal discharge accompanied by sneezing and rubbing the nose, with more or less loss of appetite and weight and a rather subacute or chronic course (which constitutes the large majority of cases), is caused by B. bronchisepticus; while the more acute form and the most fatal, in the majority of instances, is due to Bact. lepisepticum. This statement is not based on absolute findings, as both organisms have been encountered in various types of cases, but is the opinion of the authors, judging from the well recognized pathogenic powers of the two organisms and a general picture of all the cases they have observed taken as a whole.

It seems to be a fact, also, from a careful perusal of the literature relating to infections of the respiratory tract of rabbits, that these conditions are becoming less severe and are changing from acute general infections, with the most prominent symptoms associated with the lungs and surrounding tissues, to a more subacute and chronic condition, with symptoms referring to the upper respiratory tract only. Formerly all the epizoötics seemed to be sporadic in nature and extremely severe, while at the present time the infection appears to be constantly with us, spreading over the entire country, now and then increasing in severity in certain localities.

In this connection it might be stated that the opinion is rather general among those who have examined available lit-

erature on the subject of snuffles, that it is a form of hemorrhagic septicemia. This no doubt is explained for the reason that veterinary text-books give a prominent place to the rabbit septicemia organism in diseases of rabbits. This is probably due to the tendency of previous workers to investigate acute diseases of these animals, and give less attention to a chronic non-fatal disease as is characteristic of snuffles at the present time.

The fact should be kept in mind that certain members of the hemorrhagic septicemia group of organisms have been isolated from the respiratory tract of apparently healthy animals, and for this reason these organisms are classed with others such as the pneumococcus, for example, which is believed to become pathogenic under conditions which tend to lower the resistance of the host. The work of Davis, previously referred to, has shown that the "carrier" state in rabbits, as concerns the hemorrhagic septicemia organisms, is a condition to be reckoned with. One of the present writers (H. P. H.)[14] has called attention to the liability to obtain misleading results from rabbit inoculations, on account of the fact that the rabbits may actually be harboring the organisms at the time they are inoculated with suspected material for diagnostic purposes. In the event of the death of the rabbit, and obtaining a pure culture at autopsy, there is no way of determining definitely the actual source of the organism. The conditions under which hemorrhagic septicemia organisms are transformed from saprophytes to pathogens are probably quite varied, and in the disease being discussed, in some cases, at least, we may have a double infection to deal with, probably a bronchisepticus infection supervening upon a previous hemorrhagic septicemia invasion, or vice versa.

The organism found most frequently as a secondary invader, and which probably is responsible for the purulent condition of the nasal discharge and perhaps for the chronicity of many of the cases, was Staph. albus. This organism was observed in almost every case.

Oddly enough the streptococcus, which is usually considered a common secondary invader in all infectious processes, especially of the respiratory tract, was not present in one of

our cases. We must conclude, therefore, that the streptococcus is infrequently harbored by the rabbit and is not a factor to be reckoned with when undertaking to control diseases of this animal.

Conclusions.

1. Contagious nasal catarrh of rabbits, commonly called "snuffles," may be caused by any one of several microörganisms.

2. B. bronchisepticus is responsible for the majority of cases of the ordinary snuffles encountered in this country at the present time.

3. Bact. lepisepticum is an important etiologic factor, as it was found in a large number of cases, and is probably responsible for many of the acute types of the disease which result fatally.

4. Staph. albus, probably present in the nature of a secondary invader, was found in practically all cases with purulent nasal discharges.

5. Many other organisms, especially gas producers of the B. coli type, were isolated, but in such small numbers as to preclude their relationship to snuffles from an etiologic standpoint.

6. The streptococcus is probably not a factor to be considered in infections of the respiratory tract of the rabbit, as it was not found in a single case.

7. The disease can, in a large measure, be controlled with a vaccine composed of the three most prominent microörganisms mentioned above, especially if the conditions surrounding the animals are at all sanitary.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 244, 1920.

(Reprint from the *American Journal of Public Health*, Vol. 10, No. 3, March, 1920.)

SOME IMPORTANT FACTORS IN THE PREPARATION OF CULTURE MEDIA.

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The importance of placing the preparation of bacteriologic culture media on an accurate, scientific basis, has long been recognized. The fact that a majority of the pathogenic micro-organisms have similar food requirements makes such a step especially desirable. In spite of numerous attempts to formulate procedures striving towards uniformity, substantial progress in this direction has been slow. While it is aimed to record the biological characteristics of an organism with more or less precision, one of the most essential requirements, that of a proper cultural environment, is, in most cases, left in a state of uncertainty.

Very frequently, failure to obtain some important biochemical reaction, toxicogenicity, or even growth with an organism, is explained as an individual idiosyncrasy. As a matter of fact, the cause may be readily apparent when examination is made of the bacterial food or the method of its manufacture. It is the purpose of this paper to discuss some of the factors concerned in the preparation of culture media, with particular reference to the nutritive and toxicogenic requirements of the more common pathogenic organisms.

Urgent attention recently directed toward bacteriologic peptone by reason of the actual need for a domestic supply has shown that proper selection of this important culture medium constituent is imperative. It will be readily conceded that for the simple cultivation of many organisms, ordinary beef infusion or even beef extract is able to furnish all of the necessary food con-

*This investigator favors the steam pressure method of sterilization. High temperatures can effect undesirable chemical changes, while long continued heating may be destructive to food accessory factors. Emphasis is laid on the proper selection of bacteriologic peptone.

stituents. In fact, Davis and Ferry[1] have succeeded in obtaining growth of the highly susceptible *B. diphtheriæ* in a medium consisting entirely of an aqueous solution of amino acids, purine bases and mineral salts. For the more complex requirements, however, such as production of toxins or even in the growth of delicate organisms, bacteriologic peptone plays a specific rôle by supplying the essential accessory factors in the form of easily assimilated, hydrolyzed protein. As stated in a previous investigation by Davis,[2] which led to the development of a satisfactory bacteriologic peptone, the value of this product in bacterial nutrition is governed entirely by the presence of amino acids and other components which can be utilized by the bacteria. While some of these basic constituents are absolutely necessary for the maintenance of bacterial life and development, others in turn will be utilized, if present, for the production of toxins or certain by-products not essential to metabolism. Not only should a biologically utilizable peptone have the proper composition, but these amino acids and other components should be present in the most available form. Extended, comparative studies have also indicated that, among other factors, a peptone hydrolyzed to a much greater degree than the formerly imported product possesses increased nutritive values.

The superiority of beef infusion over beef extract for the cultivation of pathogenic bacteria, and particularly for the development of toxins, is generally admitted. The method formerly recommended of infusing the meat for 24 hours in the refrigerator with distilled water is practical only on a small scale. For operations involving large quantities of infusion, an equivalent, satisfactory product can be obtained by bringing the meat and requisite quantity of water to the boiling point in the course of an hour and a half and then expressing the liquid through flannel in a suitable press.

Regardless of which method is employed to prepare the infusion, fat should be removed as thoroughly as possible. Even the presence of small quantities is undesirable in the final broth to be employed in the production of diphtheria toxin or the propagation of certain surface growing organisms, *B. tuberculosis*, for example. Experience in the routine production of diphtheria toxin, observations on which will be communicated in a later

article, has shown that a marked diminution in the elaboration of toxin occurs in those flasks of bouillon containing only traces of fat. I am inclined to agree with the opinion of Larson, Cantwell, and Hartzell[3] regarding the influence of the surface tension of media on the growth of bacteria. It is very probable that this deleterious action of the fat is exerted by depressing the surface tension of the medium. This would cause *B. diphtheriæ* to grow beneath the surface with consequent decrease of pellicle and toxin formation.

One of the most important steps in the preparation of culture media is undoubtedly the adjustment of the reaction, which establishes the optimum concentration of H ions for bacterial growth and metabolism. In spite of the numerous and convincing data brought forward within the past few years to prove the fallacy of the "hot titration" method, this inaccurate procedure is still common practice. As is known, the committee of this association clearly states in the Bacteriological Section of the Revised Standard Methods for the Examination of Water and Sewage,[4] "that a new method of titrating and adjusting the reaction of culture media which will give a more accurate indication of the hydrogen ion concentration is desirable." The colorimetric determination of hydrogen ion concentration as proposed by Sörensen,[5] Walpole[6] and more recently by Clark and Lubs,[7] offers a rapid and reliable method for ordinary, bacteriologic, laboratory requirements. Estimations demanding greater accuracy should be carried out by the electrometric method. With the simple electrode recommended by Bovie[8] and the direct reading ionometer of Bartell,[9] the hydrogen ion concentration can be rapidly determined by the accurate gas chain method. A description of the "set up" required for such measurements is given in a previous article on hydrogen ion concentration determinations with diphtheria toxin (Davis).[10]

For a large number of pathogenic species including Bact. diphtheriæ, and the more delicate micrococci we have observed that reactions of the sterilized medium ranging between $P_H=8.0$ and $P_H=8.3$ ($C_H=1.0\times10^{-8}$ and $C_H=5.0\times10^{-9}$) give very satisfactory results. The general procedure given below, which employs the colorimetric method for checking the reaction, is followed with such modifications as will be subsequently discussed.

A. Add the peptone and salt as required to the fat-free infusion. Agitate thoroughly and heat for 15 minutes in streaming steam or bring to a boil to ensure thorough solution. Sugars for carbohydrate media should not be added at this point, but just before sterilization.

B. Remove exactly 10 cc. of the broth from A to a small (preferably 100 cc.) Erlenmeyer flask. Dilute with about 40 cc. of cold, distilled water, and add 0.5 cc. of a 1 per cent solution of phenolphthalein (in 95 per cent alcohol) as indicator. Titrate to a deep pink color against an N/10 NaOH solution, freshly prepared, when required, as an exact 1/100 dilution of a stock 10 N solution. The burette reading gives directly the amount of the strong (10 N) solution required to neutralize per liter of medium. Steam again for 15 minutes or bring to a boil, and estimate the hydrogen ion concentration.

C. For determining the actual hydrogen ion concentration, the simple "comparator" of Hurwitz, Meyer and Ostenberg[11] is employed, with standardized boric acid-potassium chloride-sodium hydroxide mixtures of $P_H=8.0$, $P_H=8.2$, and $P_H=8.4$, prepared as directed by Clark and Lubs.[7] Flat-bottomed test tubes, 17 mm. in diameter, of uniform bore, and with capacity of about 30 cc., have been found satisfactory for comparison purposes. Transfer exactly 10 cc. of the neutralized bouillon from B to one of the comparison tubes, dilute with 10 cc. of distilled water, and mix well. Remove 10 cc. of the mixture to another tube and add 0.5 cc. of an 0.02 per cent solution of phenolsulphonephthalein in 50 per cent alcohol. Prepare three other tubes containing 10 cc. respectively of the standardized $P_H=8.0$, $P_H=8.2$, and $P_H=8.4$ mixtures with 0.5 cc. of the phenolsulphonephthalein solution in each tube. The comparison technique is that described by Clark and Lubs.[7]

As a rule, the value will very closely approximate $P_H=8.2$. In the few cases where the color in the tube containing medium plus indicator is lighter than that of the compensated $P_H=8.0$ standard, N/10 NaOH can be run directly into the former tube until the desired tint is reached. Since the equivalent of 5 cc. of the medium is employed, twice the burette reading gives the amount of 10 N NaOH necessary to correct each litre of broth.

Media which are to contain sugars must be adjusted to a

higher value than $P_H=8.2$ before sterilization to anticipate the increase in the concentration of hydrogen ions as a result of sterilization. Where the amount of carbohydrate to be subsequently incorporated is small, this may be accomplished by titrating to a deeper shade with phenolphthalein in the preliminary adjustment, and then standardizing to a final P_H value greater than $P_H=8.2$. As an instance, broth for the cultivation of the influenza bacillus, which has, among other ingredients, 0.2 per cent of dextrose, should have a reaction coming within the narrow limits of $P_H=8.1$ to $P_H=8.2$. By adjusting to $P_H=8.3$, as above, the desired value is attained after sterilization.

When the medium is finally to have present a larger amount of sugar, the consequently greater increase in H ion concentration makes a different indicator than phenolphthalein necessary for the preliminary titration. The 2 per cent dextrose bouillon employed for toxin elaboration with *B. tetani* furnishes a good example. Experimentation has shown that in order to obtain the final optimum reaction value of $P_H=8.0$ to $P_H=8.2$, after sterilization, the broth before addition of the glucose must be adjusted to a P_H value of 8.6. This is easily accomplished by using 0.5 cc. of the 0.04 per cent solution of thymol sulphonephthalein recommended by Clark and Lubs,[7] as the indicator in the preliminary titration and adding the N/10 NaOH solution to a blue shade. Color comparison of the adjusted medium is then made in the usual manner with a standardized $P_H=8.6$ mixture, by using 0.5 cc. of an 0.02 per cent solution of ortho cresol sulphonephthalein indicator for both standard and broth, in place of phenolsulphonephthalein, as before. This is due to the fact that the range within which phenolsulphonephthalein is useful lies between $P_H=6.8$ to $P_H=8.4$.

The acid phosphate-glycerin bouillon recommended for the cultivation of *B. tuberculosis* is of interest in this connection. Preliminary cultivation experiments indicated that maximum growth of the organism can be obtained if the medium before sterilization has a hydrogen ion concentration equivalent to $P_H=7.2$. Using the hydrogen electrode for the "exploratory" work, a routine procedure was soon established. In this case, the preliminary titration is carried out according to the usual technique, to a deep pink shade with 0.5 cc. of the 0.02 per cent

solution of phenolsulphonephthalein as indicator. The H ion concentration of the adjusted and steamed medium is now determined colorimetrically in the "comparator" with standardized KH_2PO_4 —NaOH mixture of $P_H = 7.0$ and $P_H = 7.2$ (Clark and Lubs).[7] Very satisfactory color comparisons are obtained by adding 0.5 cc. of an 0.04 per cent solution of dibromthymolsulphonephthalein to the tubes containing both the broth and standards.

Solid media can be adjusted to definite H ion concentrations with no special difficulty. As has been pointed out by Clark and Lubs,[7] pure agar has practically no buffer effect in those ranges of reaction where it is usually employed, and its addition to an adjusted bouillon should have no appreciable effect on the hydrogen ion concentration of the latter. Agar, like sugars, can be incorporated into a medium just before sterilization. Since gelatin remains fluid at moderate temperatures, it can be added when necessary before the bouillon is adjusted. Serum, spinal fluid and other exudates which already possess an optimum H ion concentration should be added to media only after the reaction adjustment has been made.

A factor most frequently neglected in the preparation of culture media is proper sterilization. Theoretically, filtration through unglazed porcelain would be an ideal method for sterilizing culture media. On a practical scale, however, recourse must be had to some form of heat, generally flowing steam, or steam under pressure, as in the autoclave. As would be expected, the use of too high a temperature for this purpose can effect chemical changes in a medium which seriously impair its value for bacterial food. This is especially true where sugars or other carbohydrates are present, due to possible cleavages which may take place, particularly in alkaline solution.

Prolonged heating even at temperatures otherwise harmless should be equally avoided. This is especially of importance in the production of large quantities of media in bulk containers where a longer heating period is required to ensure sufficient heat penetration. A long distance recording thermometer of accepted type, which may be inserted directly in the liquid, will materially assist in determining the actual temperature. Contrary to the usually accepted belief, it appears that the deleterious action in this case is not due so much to a final increase in H ion concen-

tration, as to a possible destruction in the medium of food hormones, probably of a vitamin character. The alkaline reaction generally present would also render these compounds more unstable. Experimentation in progress on the rôle of food accessory factors in the nutrition of *B. diphtheriæ*, which will be reported upon in a subsequent article, appears to substantiate this belief.

In general, even with sugar media, autoclave heating at a temperature not exceeding 120 degrees C. and for a period not more than 30 minutes, has been found to give a more satisfactory microbial food than intermittent sterilization in flowing steam. Media having a supernatant layer of oil for production of anaërobiosis are preferably sterilized by the intermittent method. This avoids the danger of boiling over so frequently encountered when such media are heated by steam under pressure. Actual electrometric determinations of the same medium sterilized by both methods shows less increase in the H ion concentration of the final product when sterilized under pressure. Where a biological measurement is possible, as in the production of toxins, the intermittent method is more likely to give weaker products.

Summary.

Culture media preparation is discussed with special reference to the requirements of the more common pathogenic microorganisms. Importance is placed upon the proper selection of bacteriologic peptone. The value of this product in bacterial nutrition is governed entirely by the presence of amino acids and other components in a form utilizable by bacteria.

The presence of small quantities of fat in beef infusion has a deleterious action which is ascribed to a probable depression of the surface tension in the medium.

Hydrogen ion concentration of culture media is discussed with special reference to the colorimetric method of determination. A general procedure is outlined showing the application of this method and the modifications which are necessary in special cases of acid and alkaline media.

Comparison of sterilization procedures favors the steam pressure method. High sterilization temperatures can effect chemi-

cal changes in a medium, while prolonged heating even at safe temperatures may have a destructive action on the food accessory factors present.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 245, 1920.

(Reprinted from *The Druggists' Circular*, Vol. 64, March, 1920.)

NEED FOR A SETTLED POLICY IN BOTANICAL NOMENCLATURE.

OLIVER ATKINS FARWELL.

It seems to me that an editorial committee is an absolute necessity in order to procure uniformity in procedure, especially in the descriptions of drugs. This committee should formulate a definite method of procedure and a synopsis of the same should be given to all who are to prepare such monographs with the request that said method be followed without variance for any reason whatever; should the monographer fail to comply with such request, then the editing committee should revise the monograph where necessary to bring it in proper accordance with the method of procedure adopted.

PHARMACOGNOSY.

For instance, under digitalis in the Ninth Revision the description begins with length and breadth, shape, etc. Under uva ursi the description begins with marginal characters, shape, size, etc. Also under the former we find "Leaves when entire," etc., and a little further on "margins crenate"; to avoid ambiguity it would be better to start out with "Leaves, when not broken," etc.

The word "bark" when unmodified should be construed to mean the bark of the aerial portion of the plant only. For instance, in the Eighth Revision we have, under viburnum prunifolium, "The dried bark of the root." The change in the Ninth Revision to "bark" was made because the commercial drug had finally come to contain a very appreciable amount of the stem bark, and the unmodified word was used, or intended, to cover this condition, but in other places it means the bark of the aerial portions only. The change should have been made to read, "The dried bark of the stems and roots," as was done under granatum. But is such a change desirable? Would it not have been better to have left the specification as in previous editions?

The result is that stem bark is present in larger proportions than ever, often up to 50 per cent., and it conforms to U. S. P. definition as intended; but the stem bark is an inferior drug. The definition "bark of the root" should be restored and every effort made to have the commercial drug gathered to conform thereto. In many definitions we find the words "without the presence or admixture"; this is redundant. One or the other of these two words is sufficient.

BOTANICAL NOMENCLATURE.

There are two definite lines of cleavage in botanical nomenclature depending upon the "code" followed. There is the so-called American Code, the most undesirable features of which are perhaps the use of a trinomial system and the "slogan" once a synonym always a synonym. Then there is the International Code, with its purely binomial system, but with such multitudinous exceptions that it will forever prevent the accomplishment of that great botanical desideratum, a stable nomenclature. The United States Pharmacopœia is not the proper vehicle to exploit the ideas or views of any individual botanist along these lines, nor should it be made a publication to advance or advertise any particular code or modification thereof. It should steer a conservative course and adopt and rigidly adhere to one or the other of the codes above mentioned. In other words, it should follow, not lead, in matters pertaining to nomenclature. The International Code, even with its exceptions, will be the better code to adopt.

CAPITALIZATION.

Under the International Code, geographical names used as specific names are decapitalized. In order properly to conform to the laws governing syntax and grammar, this rule will necessitate the use of Latin type (small capitals), as HYDRASTIS CANADENSIS; if the ordinary English type (upper and lower case) is used, decapitalized, geographical specific names derived from generic names, such as "prunifolium" should be capitalized; this is eminently proper as they are proper names, but it has not been customary to print them thus. The entire question of capitalization or decapitalization can be readily avoided by printing, as indicated above, all binomial names in small capitals.

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 246, 1920.

(Reprinted from *The Therapeutic Gazette*, Vol. 36, No. 4, April, 1920.)

LOCAL ANESTHETICS.

By Herbert C. Hamilton.

(From the Medical Research Laboratories of Parke, Davis & Co., Detroit, Michigan.)

When a substitute for cocaine makes its appearance, the claims for its usefulness must be carefully examined and verified if possible by unprejudiced investigators.

Apothesine was introduced to the medical profession after being subjected to the most careful tests, including both laboratory tests on animals and clinical tests by competent physicians and surgeons.

These tests showed that apothesine, which was not only brought up but also born in America, is a true local anesthetic, about one-fifth as poisonous as cocaine, with no habit-forming effects, and with a potency fully equal to cocaine in some respects, although less effective in others.

Sollmann[1, 2] in a series of tests of local anesthetics seemed to demonstrate that in every respect it is less effective than cocaine. For example, Sollmann found that for terminal anesthesia, tested by intracutaneous administration, cocaine is eight times as potent as apothesine.

Other tests were applied, but only this one seems of especial value.

Following this an article was published by Hamilton[3] showing his results obtained by applying three of the methods used by Sollmann. The results he obtained can be summarized as follows, cocaine being taken as of unit value:

	Cocaine.	Apothesine.
For nerve blocking	1	1
For terminal anesthesia	1	1
For mucous membrane anesthesia	1	1/3

Sollmann[4] objected to the data by the second method since it was at considerable variance with his. He reported cocaine to be eight times as potent as apothesine by this method.[2] That his

report was based on considerable research is shown by Sollmann's statement:[4] "Before I tried apothesine I had made some 300 injections on myself, and the results were always quite definite."

No further published account of experiments to confirm his results has appeared under his name, but evidently such experiments were conducted,[5] since they were reported as follows by the Council on Pharmacy and Chemistry: "A further series of experiments were made by Sollmann to compare still further the results previously reported by him and Hamilton." These "demonstrated in every case that the efficiency of apothesine is unmistakably lower than that of cocaine, being at best one-half." While this is greatly at variance with his first results—showing a potency four times as great as before—there is no reason to doubt the authenticity of the report. The discrepancy is in part accounted for by the further statement[5] that there is "considerable variation in the susceptibility of different skin areas, especially to apothesine." The difference in susceptibility of different skin areas is a fact well known to the merest student in physiology. It is therefore surprising to note this admission from Sollmann.

In carrying out tests of local anesthetics by this method certain conditions should be observed to avoid error.

The injections must all be really intracutaneous. If the skin does not come up in each case in a typical raised wheal it is not a proper injection.

The injections should be made on another person and not on oneself. The reason for this is obvious, because the end point of the test is a finely drawn conclusion and must not be influenced by any knowledge which might be prejudiced.

The injections should not amount to more than 0.2 Cc.; the solution should be in physiological salt solution without preservative; it is preferable to test only one sample along with the standard at one time; the injections should be not more than one inch apart and preferably on the upper side of the forearm. There should be a fair agreement on tests on not fewer than three subjects; the subjects should be in complete ignorance of the identity and degree of dilution of samples injected.

Observing these precautions I have corroborated my first results,[3] the injections being made by Dr. N. S. Ferry into three subjects.

As before, the tests for degree of anesthesia were made by pricking the skin over the site of the injection with a sharp needle, and where the degrees of anesthesia from two injections were unlike I have indicated this difference by numbers of + signs.

Along with the test of apothesine is included a preliminary test of an experimental product included to show the data on a product less active than cocaine.

Subject—H. C. H.

A—Cocaine	1 :2000	+	+	+	+
B—Apothesine	1 :2000	+	+	+	+
C—Experimental	1 :2000	+	+	+	
A—Cocaine	1 :3000	+	+	+	+
B—Apothesine	1 :3000	+	+	+	+
C—Experimental	1 :3000	+	+		

Right Arm.

A—Cocaine	1 :4000	+	+	+	
B—Apothesine	1 :4000	+	+	+	+
C—Experimental	1 :2000	+	+		

Left Arm.

A—Cocaine	1 :4000	+	+	+	+
B—Apothesine	1 :4000	+	+	+	
C—Experimental	1 :2000	+	+		

Subject—Dr. L.

A—Cocaine	1 :3000	+	+	+	+
B—Apothesine	1 :3000	+	+	+	+
C—Experimental	1 :3000	+	+	+	
A—Cocaine	1 :4000	+	+	+	+
B—Apothesine	1 :4000	+	+	+	+
C—Experimental	1 :2000	+	+		

Miss B.

A—Cocaine	1 :3000	+	+	+	+
B—Apothesine	1 :3000	+	+	+	+

Right Arm.

A—Cocaine	1 :4000	+	+	+	+
B—Apothesine	1 :4000	+	+	+	+

Left Arm.

A—Cocaine	1 :4000	+	+	+	+
B—Apothesine	1 :4000	+	+	+	+

In the data on Miss B. the results were complicated somewhat more than in other cases by observing a difference between the two compounds in the promptness of anesthesia and the duration. These differences were not uniform, however, since in one case

the cocaine acted more promptly and in another apothesine. Not sufficient difference in the effects was observed to lead the subject to consider one better than the other. Her conclusions were, therefore, in accord with previous data.

To indicate degrees of anesthesia by the number of + signs is largely a matter of convenience in order to select for the next series of tests the dilutions of sample and standard which should give equal anesthesia. It is a practice commonly followed in biological assaying. In the above tests apothesine and cocaine continued to have practically equal efficiency and required no change except to dilute further to see if at any point the efficiency of one of them fell off compared to the other.

There was no attempt made to obtain final results on the experimental product.

It is improbable that any synthetic substance can be prepared which will equal the quick penetrability of cocaine into mucous membrane. This fact, however, should not inhibit the use of other local anesthetics if the amount and the strength of an effective solution is not poisonous and causes no untoward symptoms.

Apothesine is one-third to one-fourth as effective as cocaine for mucous membrane anesthesia. The reason is that it is absorbed more slowly and destroyed more rapidly than cocaine. Clinicians have observed no untoward effects from apothesine when used for this purpose, and in fact question whether the ratio should not be one-half instead of one-third. The factor of toxicity is negligible in any case because of the small amount absorbed and its rapid destruction.

The action of a local anesthetic on the mucous membrane cannot be measured readily. It is assumed that the relative potencies of these substances on mucous membrane will be approximately the same as their action on the frog's skin, since normally this is always moist and differs from the human skin in not being impervious to substances in aqueous solution.

The test is carried out on a frog, deprived of its brain and heart, by immersing one foot in a solution of the anesthetic for any definite period—as, for example, 2 minutes; then, after removal and drying, both feet are dipped into a dilute solution of hydrochloric acid (1/5-per-cent solution).

Anesthesia partial or complete can readily be noted by a delayed removal or failure to remove the anesthetized foot from the acid solution while the untreated foot is promptly withdrawn. A plus (+) sign indicates complete anesthesia when the treated foot is not withdrawn from the acid bath, while the untreated foot is withdrawn at once.

A plus or minus sign (±) indicates partial anesthesia shown by a delayed withdrawal of the treated foot.

Zero (0) indicates that neither foot is withdrawn from the solution, and the frog is no longer available for comparison.

The following results were obtained:

APOTHESINE.

Solution	Time immersed	Response at 2 min.	7 min.	12 min.	17 min.	22 min.	27 min.
3%	2 min.	+	+	+	+	+	0
2%	2 "	+	+	+	+	0	
1%	2 "	+	+	±	±	±	±
1%	2 "	+	+	+	±	±	0
			COCAINE				
1.0%	2 min.	+	+	+	+	+	+
0.5%	2 "	+	+	+	+	+	0
0.3%	2 "	+	+	±	±	±	0
0.3%	2 "	+	+	+	±	±	±
0.25%	2 "	+	±	±	±	±	±

The potency of apothesine is one-third to one-fourth that of cocaine applied in this way.

It seems needless to discuss the M. L. D. of local anesthetics, for it is a well-known fact that deaths from their use are not because of a near approach to that dose in clinical or dental practice, but to certain idiosyncrasies or to the previous condition of the patient.

In other words, with the exception of cocaine, the commonly used local anesthetics are applied in doses relatively minute in comparison to the M. L. D.

The following experiment readily demonstrates the relative toxicities of cocaine and apothesine on guinea-pigs. While the use of pigs for such experiments has been decried by some who contend that the cat more nearly than the rodent resembles the human in its response to the series of drugs,[6] it should be observed

that the purpose of such experiments is not primarily to establish any definite dosage for clinical use, but to compare a new local anesthetic with cocaine under exactly the same conditions.

It should be noted further that where guinea-pigs are available for such work they are usually selected because they are more convenient in every respect.

Apothesine in 10-per-cent. Solution.

Injected subcutaneously in doses calculated on the kg. weight of the pigs.

0.350	0.15	Recovered
0.345	0.20	"
0.420	0.25	Dead
0.370	0.25	"
0.390	0.20	Recovered

Cocaine in 2-per-cent. Solution.

0 385	0.03	Recovered
0.410	0.04	"
0.445	0.05	Dead
0.430	0.045	"
0.040	0.045	Recovered
0.380	0.5	Dead

M. L. D. Apothesine 0.25
" Cocaine 0.05
Ratio of toxicity, 1/5.

It will be observed that the results of these tests differ materially from those recorded by Eggleston and Hatcher[6] (p. 444), where it is shown that apothesine is three-fourths as toxic as cocaine, while my results show it to be one-fifth. Is the difference due to the technique of the operator, the animal employed, or to the method of administration?

The question of the minimum lethal dose is open to different interpretations. Some select a method similar to that used in clinical practice for the drug to be tested, while others select the method which kills with the smallest dose and most quickly. Eggleston and Hatcher[6] (p. 443) selected the method which determines the maximum toxicity "after very rapid intravenous injections of concentrated solutions." Why? In view of one of their conclusions (p. 480), that "the toxicity of the local anesthetics for the cat after subcutaneous injection has been shown to depend upon the ratio between the rate of absorption and that of elimination," their selection of the intravenous route seems inexplicable.

The selection of this method is logical only if the drug so tested is to be used in this way. But local anesthetics are never used intravenously, are never used in strong solutions, and are never injected rapidly.

Another reason against the selection of this method for comparing apothesine and cocaine is that they differ so greatly in the rate of destruction in the body; the former is destroyed very rapidly and cocaine very slowly[6] (pp. 460 and 463).

This is the important factor which accounts for the greater difference between their toxicities by subcutaneous than by intravenous administration.

Eggleston and Hatcher recognize this factor when they say: "The toxicity of the local anesthetics for the cat after subcutaneous injection has been shown to depend upon the ratio between the rate of absorption and that of elimination."

They supply data which very nicely illustrates the point. In one experiment[6] (p. 453) they injected a cat with 20-mg.-per-kg. doses of apothesine repeated every fifteen minutes until 190 mg. per kg. had been injected in 2¼ hours. No serious effects followed, the only observable effect being "a slight motor excitation," which might be expected after nine hypodermic injections.

In another experiment[6] (p. 453) a cat was injected with 10-mg.-per-kg. doses of cocaine at 15-minute intervals until 50 mg. per kg. had been injected. The effects were as follows: "Very hyperexcitable, almost in convulsions . . . clonic spasm followed by tetanic spasm" over a period of a half-hour. Four hours afterwards the animal had not recovered; it was still "depressed." This cat got about one-fourth as much cocaine as the other got of apothesine, and almost died, while the apothesine cat was scarcely affected.

From their data quoted above, we note that 190 mg. of apothesine per kilo caused no unpleasant symptoms, while 50 mg. of cocaine almost caused convulsions, and the cat was greatly depressed for at least six hours. The reason for this is summed up in their further statement that apothesine is destroyed in the body at the rate of one fatal vein dose every fifteen to twenty minutes, while in the case of cocaine one and one-third times the fatal vein dose is not destroyed in twenty-four hours.[7]

From this same data another striking conclusion may be

drawn. According to the Council,[5] "when toxicity tests of local anesthetics are made on cats these animals seem to respond to the drugs in a manner more closely approximating humans."

If a cat can be given 20-mg.-per-kg. doses of apothesine nine and one-half times in two and one-fourth hours, or a total of 190 mg. per kg., on the same basis a 70-kg. human could safely be injected with 21 grains every fifteen minutes, or 200 grains in a period of two and one-fourth hours. This leads to the logical deduction that the toxicity of a local anesthetic such as apothesine, which is rarely given in doses to exceed 5 grains, may be safely ignored when considering its properties.

Surely the rate of absorption and elimination is more important than the absolute dose, so why not use the data available? Ostensibly because[5] "one cannot reliably estimate the degree of clinical danger on animals."

This latter statement is not, however, in accord with a further statement, as follows:[5] "It is a suggestive fact that the more toxic of local anesthetics as shown by tests on cats have been found the most dangerous in clinical use."

Cocaine is near the top of their list, procaine at the bottom, but Hatcher and Sollmann have had reported to them four cases of procaine poisoning and five cases from cocaine[6] (p. 437). No other data have been published by these investigators to substantiate the statement.

The authors gave experiments where adrenalin was used with the local anesthetics intravenously and their conclusion that[6] (p. 486): "The simultaneous injection of epinephrin (adrenalin) with the local anesthetics materially reduces the toxicity, but this reduction is much less marked in the cases of cocaine and holocaine than with the other members of the series, and is referable to their much slower 'essential elimination.' "

Stripped of its peculiar verbiage this means that in the case of cocaine, elimination could not keep pace with even the delayed absorption, while in the case of apothesine because of the delayed absorption elimination kept pace until the M. L. D. was doubled.

This point has also been observed by Hamilton,[8] who explains its action by the intravenous route differently. The action of adrenalin on the heart is exactly the reverse of that of apothesine —that is, a strongly tonic action—so by the proper timing of the

adrenalin dosage it is possible to have very little depressant action on the heart from a dose of apothesine even when administered intravenously.

On the other hand, the value of adrenalin in this mixture when used subcutaneously is correctly stated by Hatcher and Eggleston to be a material delay in absorption which permits the elimination or destruction to keep pace with it.

When the two are injected subcutaneously as in clinical and dental practice, the constriction of the veins and arteries by the adrenalin prevents or retards the absorption of the drug, lowering its toxicity, and further, by this same action, adrenalin prolongs the anesthesia and tends to make weaker solutions more effective.

The use of an active member of the digitalis series also acts to reduce toxicity by its tonic action on the heart[7] (p. 480), for the same reason and in the same way as in the case of adrenalin, when the latter is applied intravenously.

From the foregoing data partly prepared for this paper, and by selection of data published by Sollmann, Eggleston and Hatcher, it is evident that:

First, the relative toxicities published by Eggleston and Hatcher have no relation whatever to the relative safety of cocaine and apothesine in clinical or dental practice.

Second, the same may be said of the data on toxicity prepared for this paper, which shows that cocaine is five times as poisonous as apothesine, since the M. L. D. is never approached in dental or clinical practice.

Third, the safety of apothesine in comparison with cocaine is shown by Eggleston and Hatcher in the experiments, where no serious results followed the administration of doses of the former, while one-fourth as much cocaine almost killed the cat.

Fourth, the efficiency and safety of apothesine is increased by the addition of adrenalin, because of delaying absorption, and by the addition of digitalis because of its tonic action on the heart.

Fifth, for two of the commonest uses of a local anesthetic (for terminal anesthesia and nerve blocking) apothesine is as potent as cocaine.

Sixth, it is an effective, non-toxic, non-habit-forming local anesthetic, especially valuable for terminal anesthesia and nerve blocking.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 247, 1920.

(Reprinted from the *Journal of the American Pharmaceutical Association,* Vol. IX, No. 5, May, 1920.)

THE GERMICIDAL VALUE OF MERCURIC IODIDE ALONE AND ASSOCIATED WITH SOAP.*

By Herbert C. Hamilton.

Mercury salts are well recognized as germicidal agents, but the different salts differ widely in germicidal power, depending on several factors. The acid radical with which they are combined, the substances with which they are associated, and the conditions of the test are all highly important factors in estimating this value.

In one case, that of mercuric iodide, it is essential that the soluble double salt of mercuric potassium iodide be prepared. The solvent probably does not in any way enhance its value except in making a soluble compound. This is apparently proved by the fact that when molecularly combined, one molecule of the mercury salt with two of potassium iodide, the resulting compound is not affected by further additions of the latter.

By the Hygienic Laboratory Method[1] the values of three of them are as follows:

Mercuric Iodide	5000
Mercuric Chloride	1000
Mercuric Cyanide	125

These values, however, are not absolute even by one method, since variable results are often found when no apparently different technique is applied.

These values, further, are usually very greatly lowered when any medium containing a greater proportion of nutrient material is used. Using plain bouillon containing very much more beef extract and peptone, the coefficients are usually reduced to about one-third the above values.

*Read before Scientific Section, A. Ph. A., New York meeting, 1920.

The term phenol coefficient, therefore, is meaningless, without the qualification which specifies the nature of the medium and the organism, the two essential factors which differentiate the different methods commonly applied.

A recent publication by Widman[2] summarized some experiments in which the phenol coefficients of two germicidal soaps and the U. S. P. Liniment of Green Soap were shown to be so nearly alike that there appeared to be no advantage in using a germicidal soap.

This is also in accord with a statement of Rosenau.[3] "Medicated soaps are for the most part a delusion and a snare so far as any increased germicidal action is concerned; in fact, the addition of phenol and other substances which have the property of combining with soap seems actually to diminish the disinfecting power of that substance." In a later publication,[4] however, he added the statement, "An exception seems to be the soap devised by McClintock in which a mercury salt exists unchanged and active."

Rosenau's statement is corroborated by Stassano and Gompel,[5] who compared three of the salts of mercury and found the iodide to be about 10 times as effective as the chloride, using the same organism—Staphylococcus. While this was mercuric iodide without the soap, the results should not be greatly different when the germicide is incorporated with a good soap base.

The soap referred to by Rosenau was one of the soaps used by Widman, and his very low results led to some further investigations.

While there is apparent reason to doubt his statement that a soap with a Hygienic Laboratory Phenol Coefficient of not less than 30 could under any circumstances be found with a coefficient less than 1, the character of the medium and the selection of the organism can greatly influence the results of an assay.

I have since then tested this Mercuric Iodide soap on three occasions, using each time a different strain of the organism he selected, *Staphylococcus aureus*, and obtained three different results, coefficients of 8, 26 and 33. In each case the work was carefully carried out and checked.

Under normal conditions of testing, namely, the Hygienic Laboratory Method, the coefficients of Mercuric Iodide and the 1 per cent Mercuric Iodide Soap are in proportion to their content of the active agent. For various reasons, mostly undetermined, this is by no means invariable, but it is approximately true in most cases and may be regarded as a general statement.

This indicates that under the conditions imposed, the soap is merely a vehicle and adds nothing to the actual germicidal value of the agent. As a vehicle, however, it has obvious advantages, such as its cleansing action, and its alkaline solution. Using *Staphylococcus aureus* as the test organism, however, quite different results were obtained, as follows:

Mercuric Iodide	1100
Germicidal Soap (containing 1% Mercuric Iodide.)	33

In this case, the mercuric iodide, associated with the soap, is 3 times as effective as mercuric iodide alone.

In another test, with a different strain of organism and a different culture medium, careful tests carried out showed results as follows:

Mercuric Iodide	86
Germicidal Soap (containing 1% Mercuric Iodide.)	26

This result was very surprising and must be regarded with suspicion, although carried out three times.

In both these cases, therefore, the action of the soap is to enhance the value of the agent very materially.

This combination has a number of advantages not possessed completely by either alone.

Soap, although for actual disinfection practically devoid of value,[6] is capable of bringing about a surprisingly high reduction in a bacterial count because of its detergent action. An efficient disinfecting agent associated with it, therefore, possesses a combination of valuable properties. This agent, however, must be high in intrinsic value, on account of the low solubility of the soap. A soap solution, as ordinarily obtained in lather, is not stronger than 1 in 100; the agent must therefore be of sufficiently high value that this further

dilution will still be effective. For example, suppose 5 per cent phenol were present in the soap, the dilution of phenol in the lather would be 1 in 2000, a dilution devoid of any germicidal value.

Another advantage possessed by the association of an active agent with soap is the fact that the solution is slightly alkaline, and as such it promotes penetration both through the fatty film on the skin and into denuded tissue.

Macfarlan[7] summarizes a number of advantages corroborating my own experiments, showing that mercuric iodide has a truly remarkable value in diseases of the skin and mucous membrane.

Incorporating a definite proportion of sodium bicarbonate readily overcomes any corrosive action on metals. This again brings out the fact that the value of a germicidal agent may often be greatly increased by association with other substances.

The object of this paper is to emphasize three points:

1st. The incorporation of an active germicidal agent with soap, if there is no combination which interferes with either substance, often enhances the values of both.

2d. Germicidal experiments should not be summarized in terms of phenol coefficients without specifically stating in what respects the assay methods differ from the accepted methods of obtaining the phenol coefficient.

3d. Staphylococcus is a logical organism to use for such tests, but on account of the variation in resistance of different strains of the organism, no result can be taken as final and invariable.

PROTOCOL OF GERMICIDAL TESTS.

Sample—Germicidal Soap.
Method—A. P. H. A.
Organism—*B. typhosus*.

Sample.	5.	20 min.	Phenol	5.	20 min.
1-2000	—	—	1-80	—	—
2500	—	—	90	—	—
3000	+	—	100	—	—
3500	+	—	110	+	—
4000	+	+	120	+	—
1-2700	—	—	1-100	—	—
3000	+	—	110	+	—
3300	+	—	120	+	—
3600	+	—	130	+	—
4000	+	+	140	+	—

Coefficient 26.4.

Sample—Germicidal Soap.
Organism—*Staph. aureus.*
Medium—Hygienic Laboratory.[1]

Soap	2½.	15 min.	Phenol.	2½	15 min.
1-400	+		1-50	—	
600	+		60	—	
800	+		70	—	—
1000	+		80	+	—
1200	+	+	90		—
1400	+	+	100		+
1600		+			
1800		+			
1-100	—		1-50	—	
200	—		60	—	
300	—		70	—	—
400	+		80	+	—
500	+	—	90		—
600	+	—	100		+
800		—			
1000		+			
1200		+			
1400		+			

$$\text{Coefficient} = \frac{300}{70} + \frac{900}{90} = 7.$$

[1]Adjusted to + 1 5 acidity.

Sample—Green Soap.
Organism—*Staph. aureus.*
Medium—Plain Bouillon.

Soap	2½.	12 min.	Phenol.	2½.	15 min.
1-20	+		1-70	—	
30	+		80	+	—
40	+		90	+	—
50	+	+	100	+	+
60		+	110		+
70		+			
80		+			

Coefficient less than 0.3.

Organisms—*Staphylococcus aureus.*
Medium—Plain Bouillon.
Substance—Mercuric Iodide as Mercuric Potassium Iodide.

Mercury Comp.	5.	20 min.	Phenol.	5.	20 min.
1-80000	—	—	1-80	—	—
100000	+	—	90	+	—
150000	+	+	100	+	+
200000	+	+			
250000	+	+			

Coefficient 1100.

Organism—*Staphylococcus aureus.*
Medium—Plain Bouillon.
Substance—Mercuric Chloride Phenol.

$HgCl_2$	5.	20 min.	Phenol.	5.	20 min.
1-10000	—	—	1-80	—	—
50000	—	—	90	+	—
20000	—	—	100	+	+
30000	+	—	110	+	+
40000	+	+			

Coefficient 333.

Organism—*Staphylococcus aureus.*
Medium—Plain Bouillon.
Substance—Germicidal Soap.

Soap	5.	20 min.	Phenol.	5.	20 min.
1-2000	—	—	1-80	—	—
2500	—	—	90	+	—
3000	+	—	100	+	+
3500	+	+	110	+	+
4000	+	+			

Coefficient 33.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 248, 1920.

(Reprinted from *The Journal of the American Veterinary Medical Association*, May, 1920, Volume LVII, New Series Volume 10, Number 2.)

STUDIES ON ANTHELMINTICS.

VIII—Some Experiments with Fluid Extracts.

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In a previous paper (Hall, 1918) the writer has made the following statements in regard to fluidextracts as anthelmintics:

"Fluidextracts or other preparations using alcohol as a solvent for active anthelmintic ingredients are frequently unsuitable as anthelmintics.

"Experiments in this laboratory on a number of such preparations indicate that there are good objections to some of these preparations. In the first place, the very fact that alcoholic preparations are adapted to the production of rapid systemic effect—the effects one wishes to avoid in using the characteristically toxic group of drugs known as anthelmintics—is one reason why they are unsuitable as anthelmintics. These alcoholic preparations are often rapidly absorbed, largely in the stomach and duodenum, occasioning more or less irritation at the point of absorption and producing systemic effects of a more or less toxic nature. The considerable and rapid absorption leaves a comparatively small amount of drug available for actual anthelmintic action, and by the same token leaves the minimum of drug that could possibly be removed by purgation, after exerting its anthelmintic effect. In the writer's opinion, some alcoholic preparations of anthelmintics are distinctly dangerous to the host animal and relatively ineffective against parasites, and this opinion is substantiated by quite a number of experiments in this laboratory."

The object of this paper is to detail some of these experiments on fluidextracts as anthelmintics.

The fluidextracts tested were those of kamala, chenopodium, balsam poplar buds (balm of Gilead), caulophyllum (blue cohosh), and spigelia (pink root).

*Resigned March 27, 1919.

Kamala in powder form is well known as a very satisfactory tæniafuge and is one of the two remedies used in removing the common liver fluke from sheep. Tests of the fluid extract were made with 3 dogs as shown in the following table:

Dog No.	Weight	Dose	In Water	Worms Passed				Worms Postmortem				Digestive Tract
				Ascaris	Hookworms	Whipworms	Tapeworms	Ascaris	Hookworms	Whipworms	Tapeworms	
	Kilos	Mils	Mils									
24	7.5	8	24	1	0	0	2	0	2	2	2	Normal
48	11	8	24	0	0	0	0	0	1	0	37	Normal
206	9	16	None	0	0	0	0	2	0	0	10	Gastric hemorrhage

The treatment removed 1 of 3 ascarids (33 per cent), none of 3 hookworms (0 per cent), none of 2 whipworms (0 per cent), and 2 of 49 tapeworms (4 per cent). This result is sufficiently inferior to the results we can depend on attaining with other drugs to warrant the belief that this preparation cannot be regarded as a valuable anthelmintic.

In small doses (8 mils) the drug was uninjurious to the digestive tract, but in larger dose (16 mils) post-mortem examination showed hemorrhages in the stomach.

Chenopodium in the form of oil of chenopodium is the most valuable all-around anthelmintic that we know of, being the best for use in single-dose treatment against ascarids and giving better results in three doses at hour intervals for removal of hookworms than thymol in therapeutic dose gives. Tests of the fluidextract were made with 3 dogs as follows:

Dog No.	Weight	Dose	In Water	Worms Passed				Worms Postmortem				Digestive Tract
				Ascaris	Hookworms	Whipworms	Tapeworms	Ascaris	Hookworms	Whipworms	Tapeworms	
	Kilos	Mils										
55	18	4	12 mils 01. ric. 30 mils	1	0	0	0	3	0	33	80	Inflamed
56	14	4	12 mils 01. ric. 30 mils	0	0	0	0	22	1	0	19	Inflamed
59	4	2	6 mils 01. ric. 20 mils	0	0	0	0	1	1	1	0	Gastric hemorrhage

The treatment removed 1 ascarid out of 27 (4 per cent) and none out of 2 hookworms, 34 whipworms, and 99 tapeworms (0 per cent). In view of the high anthelmintic efficacy of oil of chenopodium, there would appear to be no reason for using a fluidextract which shows so little efficacy as an anthelmintic.

In all cases the digestive tract was inflamed or showed gastric hemorrhage, as would be expected from the fluidextract of chenopodium, since we know that the oil of chenopodium acts as a gastro-intestinal irritant, and alcohol would probably increase the rate of absorption.

The fluidextract of balsam poplar buds was tested as follows:

Dog No.	Weight	Dose	In Water	Worms Passed				Worms Postmortem				Digestive Tract
				Ascaris	Hookworms	Whipworms	Tapeworms	Ascaris	Hookworms	Whipworms	Tapeworms	
	Kilos	Mils										
174	13.5	4	30 mils	0	0	0	0	0	0	0	0	Normal
179	12	4	None	0	0	0	0	10	0	8	0	Normal
176	11.5	10	None	55 or 18	0	0	0	0	0	0	0	Normal

Dog No. 176 and another dog in a different experiment escaped from their cages on one occasion, making it impossible to say which one passed a number of worms that were found outside of the cages, hence the two figures given for ascarids passed. However, the experiment shows that in doses of 10 mils, undiluted, the fluidextract of balsam poplar buds is 100 per cent effective against ascarids, whereas in doses of 4 mils, undiluted, it is 0 per cent effective against ascarids.

The digestive tracts were normal in all 3 dogs. It should be said in comment that the fluidextract of balsam poplar buds contains a large amount of difficultly soluble material, oleoresins and resins, which material is promptly thrown out of solution on the addition of a small amount of water or on contact with the buccal mucosa. This comparative insolubility probably accounts for the fact that the drug causes no injury to the digestive tract and also for the necessity for large doses.

The following tests were complicated in a way that makes tabulation unsatisfactory, so they are given in detail:

Dog No. 105, weighing 7.5 kilos, was given 7.5 mils of fluidextract of spigelia and senna in 15 mils of water. The next day the dog passed 1 ascarid. No more worms were passed until the fourteenth day, when 5 ascarids were passed. While the passage of these worms at this late date might be partly due to toxic effects of the anthelmintic, which weakened the worms in the first instance, the connection is too uncertain and too tenuous. Ordinarily our experiment animals are killed on the fourth day, as our findings (Hall, 1918) show that about 98 per cent of the worms passed by dogs after an anthelmintic come away in the first 4 days after treatment, the remaining 2 per cent coming away on the fifth to the seventh day. It is undeniable that an anthelmintic might weaken a worm to the point where it would succcmb to unfavorable conditions 2 weeks later, but such action must be disregarded in a consideration of anthelmintics. To merit consideration as an anthelmintic in experiments on dogs, a drug must furnish of itself such unfavorable effects on worms as to bring them away within a week, and the big majority of worms, in fact, actually do come away from dogs within the first 24 hours after the administration of the anthelmintic. In the case of dog No. 105, the animal had 45 ascarids and 10 *Dipylidium* post-mortem, showing an efficacy of 2 per cent against ascarids and 0 per cent against *Dipylidium*. The digestive tract showed a moderate degree of inflammation.

Dog No. 170, weighing 4.5 kilos, was given 1 mil of fluidextract of caulophyllum (blue cohosh) in 3 mils of water. During the next 5 days the dog passed 5 ascarids. On the fifth day the dog was given 4 mils of fluidextract of balsam poplar buds in 12 mils of water. The dog was dead the next morning. Postmortem examination showed 64 ascarids in the large intestine and cecum. These must be credited to the anthelmintic action of the balsam poplar buds, and the number would presumably have been greater if the dog had not died too soon after treatment to permit the anthelmintic to display its total efficacy. However, this dog had in the small intestine 1,985 ascarids, most of them very young worms, and 1 hookworm. There was some inflammation and hemorrhage in the digestive tract. The early death of the dog and the other complications here make it difficult to draw conclusions.

SUMMARY.

The low tæniacidal value of fluidextract of kamala as compared with the high tæniacidal value of powdered kamala, and the low ascaricidal value of fluidextract of chenopodium as compared with the high ascaricidal value of oil of chenopodium, bear out the statement that fluidextracts are frequently unsuitable as anthelmintics. Fluidextract of spigelia and senna promises little of value as an anthelmintic, and this is in agreement with Foster's findings, published by Hall and Foster (1918). Fluidextract of balsam poplar buds may prove to be effective against ascarids, and uninjurious, when taken in large doses, but large doses of this drug, with the precipitation of the resinous content on the buccal mucosa, are resented by dogs and would not be very attractive to man. Fluidextract of caulophyllum did not receive sufficient test to draw conclusions on, but in the dose used it was not very effective.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 249, 1920.

(Reprinted from *The Journal of the American Veterinary Medical Association*, June, 1920, Volume LVII, New Series Volume 10, Number 3.)

SNUFFLES (CONTAGIOUS NASAL CATARRH) OF RABBITS: ITS ETIOLOGY AND TREATMENT.

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Within recent years the rabbit raising industry in the United States has become one of immense proportions. There are quite a number of reasons why rabbit breeding and raising appeals to the average individual, and as a consequence we find a number of rabbitries in practically every locality, in our large cities, small towns, and even in the country districts. The utilization of rabbits as a source of meat has been a common practice in some foreign countries for many years, but only within recent times has this phase of rabbit raising attracted attention in this country. Rabbits were raised to be sold as pets, or for use in laboratories as experiment animals.

Rabbits are very prolific breeders and the young rabbits from good stock mature very rapidly. They require a minimum amount of space, much less than chickens, and are easier to raise. In fact, it is somewhat surprising how thrifty some rabbits will be in what we would ordinarily consider cramped quarters. When properly cooked, rabbit meat is very palatable, and it might easily pass for chicken. The pelts have a market value if carefully removed and dried.

At the present time rabbit breeders are experiencing considerable trouble with a disease which is generally called "snuffles." It is also designated "cold," "catarrh," "distemper," and even "flu." It is characterized by disturbances of the respiratory tract, marked chiefly by a nasal discharge which varies in amount, color and consistency in various stages of the disease. Affected rabbits sneeze at frequent intervals, resulting in the discharge of considerable nasal mucus. There is apparently some irritation of the

nasal passages, as the sick rabbits are seen to rub their noses with their front paws, evidently to alleviate this irritation. As a result of using the paws for rubbing the nose, the former are much soiled and the fur badly matted by the nasal discharges. Other parts of the body may become soiled, resulting in an untidy appearance. On the other hand, some rabbits, even though affected for months, will maintain a sleek, thrifty appearance.

Snuffles is not usually a fatal disease. Some rabbits will make spontaneous recoveries. A second attack, following a complete recovery, is rare. In some cases the disease persists for six months, if untreated. There will be intervals when the symptoms subside and almost disappear, only to recur a few days later. Damp, humid weather seems to aggravate the condition.

There seems to be no age when rabbits are most susceptible, although the disease and the conditions attending it are such as to make it appear that young rabbits contract the disease most readily. Mature rabbits coming from a rabbitry where the disease has not existed to one where the infection is prevalent frequently show symptoms a short time after introduction. From observations thus far made under natural conditions, and not controlled by artificial exposure tests, the incubation period varies from one to three weeks. The disease is not rapidly contagious. Young rabbits from the same litter, even if kept together and equally exposed to infection, will not all develop the disease at the same time, but will show first symptoms at variable intervals.

There appears to be quite a little difference of opinion among rabbit breeders upon several points in connection with snuffles. Whether the disease called snuffles is one distinct pathological entity, or whether there are two or more diseases with about the same train of symptoms, is open to discussion. Some rabbits will die rather suddenly, after having the disease for some time. These rabbits show acute symptoms, such as marked depression and quickened breathing, and frequently die within 24 to 36 hours. Usually a pneumonia will be found at autopsy.

Rabbits killed while suffering from chronic snuffles, for the purpose of studying the pathological changes incident to the disease, show practically nothing of significance except in the nares and nasal sinuses. Here we find the nasal mucous membrane congested and covered with a secretion varying in consistency from a

sero-mucous to a muco-purulent nature. In the sinuses proper this secretion is frequently found in an inspissated condition, forming casts. The lower respiratory tract shows little or no change beyond congestion, except in those acute cases, already referred to, where a pneumonia sometimes supervenes.

Ferry and the present writer (1) have made a study of the bacteriology of the disease. These investigations would seem to indicate that more than one microörganism plays some part in the affection. Cultures made by introducing a sterile swab into the nostrils of sick rabbits, or direct from the nasal passages, trachea and heart blood of rabbits dead of the disease, or killed for autopsy purposes, have yielded the following organisms most consistently: *Bacillus bronchisepticus, Bacterium lepisepticum, Staphylococcus albus.* Besides these three organisms various others are found in the nares, but without any regularity. They are not found in the trachea or upper nasal passages, but only in those rabbits examined alive, by passing a swab into the nostrils. It is quite likely that all such organisms are accidental invaders, probably having been inhaled with dust and of little importance from an etiological standpoint.

The first-named organism, *Bacillus bronchisepticus*, is now regarded as the causative agent of canine distemper, based on the investigations of Ferry (2), McGowan (3), and Torrey and Rahe (4). The organism has been isolated from dogs suffering with distemper, pure cultures being obtained from the respiratory tract and from the blood. In a more recent publication Ferry (5) states: "It is also of interest to know that this microörganism is known to produce a disease somewhat similar to distemper in other animals, such as the rabbit, guinea pig, cat, monkey, ferret, and likewise in a few cases in the human subject." Hoskins and Stout (6) have found the same organism to be the cause of a respiratory infection in white rats, having somewhat the same clinical picture as snuffles in rabbits.

The next most frequently found organism is *Bacterium lepisepticum*, a member of the hemorrhagic septicemia group, previously isolated by several investigators and given different names. It is of more than passing significance that the rabbit septicemia organism should be found at this time to play a part in a widespread disease of rabbits, when hemorrhagic septicemia of cattle, sheep

and hogs is receiving so much attention. Just as these organisms are found in the tissues of apparently healthy cattle, sheep and swine, so do we find them in apparently healthy rabbits. The assumption is that they become pathogenic under certain conditions, not well defined, but in a general way tending to lower the resistance of the host harboring the organisms.

The third organism found quite regularly in the nasal passages is *Staphylococcus albus*. It is quite likely that this organism is a secondary invader, but probably responsible in no small degree for the chronicity of the disease. In the very early stages the nasal discharge is watery. Later this becomes thicker in consistency and white, creamy or grayish-yellow in color, probably due to the invasion and multiplication of the staphylococci in the diseased tissues.

The treatment of snuffles offers a real problem. Reports received from rabbit breeders state that many and varied forms of medication have been employed, but without satisfactory results. Agents of many different sorts have been employed for internal administration, nasal sprays, and hypodermic injections. The latter include serums and vaccines of various sorts, found upon investigation to have been suggested by physicians and veterinarians, on account of the similarity of snuffles with certain other diseases, such as canine distemper or chronic nasal catarrh of man.

The primary object of this paper is to call the attention of veterinarians to the disease of rabbits which is generally called snuffles. That there is an undeveloped field in this direction cannot be questioned. Case after case has come to the attention of the author where a veterinarian had been called in by a rabbit breeder for the purpose of giving advice on the control of the disease, and almost without exception the veterinarian would say that he knew nothing about rabbit diseases—a frank confession, it must be admitted. That the rabbit breeder is anxious, even insistent, that somebody come to his assistance, is evidenced by the receipt, almost daily, of letters from different parts of the country, inquiring for some remedy for snuffles.

In the absence of any specific remedy, these rabbit breeders have tried anything and everything that overanxious friends would suggest. Some of the remedies that have been tried border on the ridiculous. The more observant breeder has noted the fact that hygienic quarters for his rabbits mean less disease. Here is a

chance for the veterinarian to be of very great assistance in the rôle of sanitarian. He can render advice on such fundamental points as feeding and housing, especially light and ventilation, and the value of regular cleaning and disinfection of the hutches. He should have a knowledge of the various breeds of rabbits and know enough, at least, not to pick up a valuable animal by the ears. Unless veterinarians do pay some attention to rabbit practice, this work will get away from them, and it will be a difficult matter to get it back. We will hear criticisms such as we heard a few years ago concerning swine practice, namely, that remedies were being sold to and used by the laity, the veterinarian being overlooked.

Due to the fact that snuffles has all the appearance of an infectious and contagious disease, and the fact that bacteriological examinations of rabbits affected with the disease, made at different places and by different workers in the past, show a somewhat constant combination of pathogenic organisms present, attention has naturally been turned to the possibilities of preventing and curing the disease with a specific biologic agent. A bacterin containing the three organisms previously mentioned has been the basis of some experiments to treat the disease. This work has been under way for some time, with rather encouraging results, and a report of the results following such treatment of the disease will be published at a later date.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 250, 1920.

(Reprinted from *The Journal of Laboratory and Clinical Medicine*, St. Louis, Vol. V, No. 9, June, 1920.)

ACTION OF CHLORETONE ON ANIMAL TISSUE.

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(From the Medical Research Laboratories of Parke, Davis & Co., Detroit, Mich.)

Trichloro-tertiary-butyl alcohol (chloretone) has a number of properties which make it valuable therapeutically, among which may be mentioned its hypnotic, anesthetic, and bactericidal properties.

It finds its most important use, however, at the present time, as a hypnotic and sedative; but its local and general anesthetic properties must not be lost sight of.

As a general anesthetic for experimental animals, where it is not necessary to recover the animal, it is an ideal general anesthetic, and it is employed for this purpose in many laboratories. When given in small doses prior to general anesthesia with ether or chloroform, in the human, it has been found that the patient requires less ether or chloroform, that the period of excitement is lessened, and that unpleasant postoperative vomiting is often lacking.

As a germicide, its aqueous solution will kill all but the most resistant spore-bearing germs, and as a mild local anesthetic it may be employed to advantage in dressing burns, abrasions, cuts, etc.

As an obtundent in dentistry, either alone or in oil, it is ideal, as evidenced by many reports.

The action of chloretone on animal tissue, however, has not been studied to any extent, and it is to this that we wish to call attention.

A number of years ago, it was found that pituitary and adrenal glands when placed in a saturated aqueous solution of chloretone (0.5 to 0.8 per cent) kept indefinitely. In fact, a number of glands in our possession have been kept for over ten years, without any apparent decomposition as evidenced by odor and gross

appearance. It was furthermore found that chloretone added to saturation in aqueous solution would prevent the development of molds, and this fact has been made use of in the preparation of aqueous stock solutions of the alkaloids and of solutions of adrenalin, ergot, pituitrin, etc.

It was thought also that the aqueous solution, or a modification of it (physiologic salt solution saturated with chloretone), *might* be of value in the preservation of not only animal tissue, but aquatic specimens (both plant and animal), museum specimens, also material for laboratory class work in high schools and colleges. Histologic studies, as will be cited later, have shown, however, that chloretone can only be employed as a differential preservative: that is to say, a preservative which leaves some tissue elements intact and allows autolysis or other changes to proceed while keeping down bacterial action without the destruction of the active constituents set free. In the investigation of the pituitary glands, for example, the glands as well as the extract may be preserved with chloretone until the finished product is obtained without harm to the active constituent, and it may well be that chloretone will find one of its many uses along this line.

Although the preservative action of aqueous chloretone solution has been known to us and employed by us in the preservation of glands and gland extracts from bacterial decomposition, for a long time, no systematic experimental work has been carried out along this line to show its general action on animal tissue. Our object in presenting this preliminary paper is not to present an exhaustive article, but merely to draw attention to the effect of chloretone in aqueous solution on animal and possibly vegetable tissue.

In order to test the action of a saturated aqueous solution of chloretone on animal tissue, the following organs—brain, heart, kidney, liver, spleen, testicle—were removed as quickly as possible from the animal after death, cut into small pieces and distributed among seven sets of bottles. Each bottle contained about 140 c.c. of saturated solution of chloretone (0.5 to 0.8 per cent), and was tightly corked to prevent the chloretone from volatilizing. To keep the water and air saturated, a slight excess of chloretone crystals was usually left undissolved at the bottom of the bottle, it being assumed then that the water was sufficiently saturated.

Set "A" was placed in the incubator at 37° C.

Set "B" was placed in the refrigerator at 15° C.

Set "C" was infected with B. proteus and kept at room temperature.

Sets "D," "E," "F," and "G" were kept at room temperature.

A piece of each organ was also placed in each of two bottles, one containing only distilled water and the other 50 per cent alcohol. The sets were examined every few days and the general appearance, odor, change of color, etc., noted.

The gross changes observable after six weeks' exposure to the chloretone solution were as follows: Brain sections appeared unaltered in color but of rather softer consistency.

Heart muscle had lost its normal color, appearing pale even when cut, and more elastic than when fresh.

Kidney segments appeared of a dull uniform yellowish cast as compared to their fresh, dark-red, natural color. They were somewhat tough and elastic.

Liver portions presented a rather unusual dull orange-colored appearance confined to the surface, the interior portion retaining the original deep characteristic dark-red color. The tissue itself was remarkably soft, flabby, but tough.

Spleen sections retained their original color and appearance, although feeling more soft and spongy.

Testicular pieces were apparently unchanged in color or consistency.

When bacteriologic tests were applied at ten-day intervals, all bottled lots were found to be sterile.

In three sets molds grew in the culture media, but no multiplication had taken place in the preservation bottles. In fact, aside from a rather limited amount of débris arising from occasional handling, the liquid remained clear and bright, the tissues being visibly free from the common signs of decomposition and putrefaction.

In addition to the above records a series of observations* were made upon the histologic changes undergone by the treated tissue. Two samples of material were used: (a) liver of dog, which had been kept at 37° in sat. sol. of chloretone in water for 15 days,

*These observations were reported by C. J. Marinus, to whom we wish to express our thanks.

and (b) bovine suprarenal, which had been kept at room temperature in sat. sol. of chloretone in water for a period of years. Small portions of each tissue were placed in 10 per cent formalin for three days, dehydrated in graded alcohols, cleared in toluol, embedded in paraffin, cut 7 micra thick and stained with Delafield's hematoxylin and eosin. Control sections of formalin-fixed rabbit ovary were stained under identical conditions.

(*a*) *Dog's Liver.*—The low power section presents a foam-like appearance, due to absence of cytoplasm and nucleus. Only cell membrane remains, staining a grayish-violet, surrounding a clear space. The connective tissue stains a diffuse pink around the larger blood vessels and bile ducts. The fibrils are softened, swollen and edematous. Blood corpuscles are absent.

(*b*) *Bovine Suprarenal.*—Under the lower power the section may be differentiated into two regions, an outer cortex and an inner medulla. The cortical cells present a greenish-brown appearance and the high power shows the cells filled with coarse brown granules similar in appearance to those seen in formalin preparations. In many cells only faintly stained cell membranes remain. The typical structure of the cortical cells is faintly suggested but not definitely shown.

The medulla presents an appearance similar to that of the cortex. Each cell is distinguishable, however, although the nucleus is absent.

These facts indicate that preservation in chloretone water does not preserve cytological details. The cytoplasm and the nuclear material are completely dissolved. Only the cell outlines are preserved. Connective tissue is altered, swollen, edematous, but apparently persists. Certain lipoid granules, characteristic of suprarenal cortical cells, were found present after some years of treatment with chloretone water. This suggests the possibility that specific cell products may be preserved by the chloretone water.

Microscopically there was no evidence of bacterial action in any of the sections examined. They show, however, that the processes of autolysis are not inhibited by chloretone. The resistance of suprarenal lipoidal granules in the face of the generalized destruction of tissue substance is also a noteworthy fact.

The results obtained from our limited study are at first glance distinctly disappointing. Apparently chloretone possesses little

value as a fixative of animal tissues. The histologic evidence is conclusive on this point, so far as the selected tissues examined are concerned. This may not prove to be wholly applicable to other tissue on extended study.

But when we consider the greater value of animal tissues as holders of specific principles, we find that this substance does possess in a unique way what we are pleased to express as differential preservative value. This property, we believe, is capable of being extended to a wide range of practical applications.

The bactericidal action of chloretone is, therefore, of special interest in this connection, since there are but relatively few substances capable of favoring autolysis under ideal conditions.

CONCLUSIONS.

1. Chloretone in saturated aqueous solution exerts a definite bactericidal action at all temperatures.

2. Chloretone in saturated aqueous solution prevents the development of the common molds.

3. Chloretone solution is not suitable as a fixative for histologic materials.

4. Chloretone in saturated solution, while acting as a bactericide, does not inhibit autolytic action as evidenced by our histologic findings.

5. Chloretone solution is a desirable agent for preserving glands and gland extracts from which the active principles are to be obtained.

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 251, 1920.

(Reprinted from *The Bulletin of Pharmacy*, Vol. 34, No. 6, May, 1920.)

WHAT SHOULD BE THE SCOPE OF THE NATIONAL FORMULARY?

By Oliver A. Farwell.

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The scope of the N. F. must depend largely upon the "reason for its being." If it is to be considered as an official directory of standards for drugs not included in the U. S. P. (and as such it will be a complementary volume to the U. S. P.) and intended for the guidance of the officials who have the enforcement of the pure food and drugs act, the prohibition measure, etc., qualification for admittance must necessarily depend largely upon usage.

Certainly the standardization of drugs should not be permitted to fall into the hands of political officials who would determine the standards haphazardly. On the other hand, the N. F. might, and probably would, command a higher respect if qualifications for admittance were based entirely upon therapeutic values, excluding any preparation that could be used as an alcoholic beverage.

The latter course probably would be the better one, especially if it will help to throw into the discard, there to keep company with the old remedies of the ancient herbalist, many if not all of the preparations that are without therapeutic value. To give official backing to remedies that are inert and valueless, no matter how much they may be in "general use," smacks of charlatanism.

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 252, 1920.

(Reprinted from *The Journal of Laboratory and Clinical Medicine*, Vol V, No. 9. June, 1920.)

HEMOSTATIC AGENTS.

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The blood is the agent of metabolism in that it serves as the medium by which nutritive and other substances are conveyed to different parts of the body and waste or noxious substances are removed. The property therefore of remaining liquid in the vascular system is of such vital importance as to overshadow another property that is little appreciated—the property of clotting spontaneously when the blood escapes from the severed vessels either because of wounds or disease. In a few minutes after normal blood has escaped from lacerated tissues it is gradually transformed into a gelatinous mass. It is the formation of this clot that checks the hemorrhage in small injured vessels which would otherwise bleed continuously.

Ordinarily most hemorrhages of moderate degree can be controlled with the usual measures of pressure and dressings. However, even in persons having a normal healthy coagulability of the blood, lacerations of tissue occur in which such ordinary measures of control fail, entailing a great loss of this vital fluid. Instances of this type are hemorrhages due to oozing after tonsillectomies, prostatectomies, nasal operations, and operations in vascular regions. It is evident that an increased coagulability of the blood in these cases is greatly to be desired, since it will increase the automatic control of hemorrhage normally possessed by the organism.

A tendency to hemorrhage due to hemophilia or to hemorrhagic diathesis undoubtedly renders the use of such an agent an indispensable procedure.

The prompt clotting of the blood under normal conditions is apparently due to the fact that the action of antithrombin, maintaining the fluidity in the vessels, is arrested by the fluids exuding

from the lacerated tissue. These mix with the blood and initiate the process of clotting.

One of the constituents of the blood immediately concerned in clot formation is fibrinogen, which on being subjected to the action of thrombin is changed into an insoluble fibrinous or gelatinous mass called fibrin. The formed elements of the blood become enmeshed in this, and the clot results.

Thrombin as such does not exist in the circulating blood. It is derived from a mother substance conveniently designated as prothrombin, which is quickly changed to thrombin in shed blood by the thrombokinase derived from the blood platelets and the lacerated tissues, in the presence of the free Ca-ions of the plasma.

In the normal subject therefore the natural process can be depended upon except when considerable laceration occurs or when it is an internal injury. Under these latter conditions a shortening of the coagulation time is desirable and may even be vitally necessary.

Two substances suggest themselves for the purpose—the thrombokinase that may be derived from blood platelets and from tissue juices, and a substance to neutralize the antithrombin action.

The former substance from whatever source derived has been variously named—the term thrombokinase being apparently most appropriate on the theory that the action has the nature of a kinetic function rather than chemical. The sources of thrombokinase are the blood platelets and tissue fluids, while a substance of a similar character known as cephalin is present in the brain. The latter seems to be exceptionally strong in kinetic power, but is applicable only for local use because of its waxy insoluble nature.

Thrombokinase is lacking in any specific action to counteract the antithrombin:[1a] it serves only to initiate the change of thrombin from the inactive prothrombin to the active form. The rôle of the antithrombin therefore seems to be of utmost importance and the minimizing of its power essential in any attempt to shorten the coagulation time of the blood. This may be brought about by the inclusion of a specific antiantithrombin.

There are certain cases, comparatively rare, in which the blood fails to coagulate or in which the process is so slow that a hemorrhage might be fatal before clotting occurs. In these cases it is demonstrated that the equilibrium between antithrombin and pro-

thrombin is at fault, because of an excess of the former or a deficiency of the latter.

The third constituent therefore that should be present in a well balanced hemostatic is prothrombin to restore the balance in the blood.

The substances previously proposed for the control of hemorrhage by changing the character of the blood, are blood serum, both liquid and powder, purified thrombin, blood platelets in both liquid and powder form and brain extract in the waxy form resulting from extraction with ether and acetone. An aqueous suspension has also been prepared by making a salt solution extract. This is for hypodermic injection.

Freshly prepared blood serum or the powder resulting from a precipitation of the thrombin in an active state has an obvious advantage as an aid in shortening coagulation time, because in this way active thrombin is apparently made immediately available. Almost equally logical is the use of blood platelets which seem to have a specific action to initiate clot formation.

Both of these classes of coagulants are normal constituents of the blood and both are immediately concerned in the process of clotting—thrombin as the agent which brings about the change of fibrinogen to fibrin and thrombokinase (from the platelets) because of its observed value in initiating the reaction by which prothrombin takes on the active form thrombin.

According to Howell[2] and others, cephalin from brain substance is more active in this respect than the disintegrated blood platelets, but such an extract is not well adapted to hypodermic administration and is particularly inadvisable for intravenous use.

No single substance has been suggested which has the power to neutralize or minimize the action of the antithrombin, nor is there available in the above list any true blood coagulant adapted to meet all the necessities of every kind of hemorrhage. Thrombin alone is immediately destroyed by antithrombin; thrombokinase cannot act on unreleased prothrombin. Coagulation time may be influenced by any of these agents to a slight extent if the substance is active, is properly applied, and is adapted to the particular case, but the highest efficiency is only rarely attainable because one or more of the above conditions is not fulfilled.

There is unquestionably, therefore, a field for a coagulant com-

bining the valuable properties of those mentioned as well as supplying the essential properties which are lacking in the above list.

It should have kinetic power supplied by an active thrombokinase; it should contain prothrombin in case this constituent is deficient; it should have a substance to minimize the action of the antithrombin and thus permit the formation of thrombin. Such an agent is hemostatic serum.[1b]

Hemostatic serum contains prothrombin, antiantithrombin and thrombokinase in a physiologically balanced solution, with a demonstrated value in shortening coagulation time when administered by any of the accepted methods. Intravenous administration is naturally the route by which quickest results are obtained.

Various methods have been suggested for standardizing blood coagulants. Of these, local application to a superficial wound and the measurement of the coagulation time of the escaping blood seems least satisfactory. Such a method is logical for adrenalin and substances which act as astringents locally on the tissues; and also substances such as the insoluble waxy material extracted from brain substance and recommended by Hess the orginator[3] for local use only.

There are several reasons why a soluble substance cannot be standardized by this method. A liquid is so promptly washed away by the current of blood that it is almost valueless locally unless applied on gauze. It should not be applied by periodic swabbing with a cotton pledget saturated with the agent[4, p. 201] since this retains the fluid and prevents close contact with the wound; it is practically impossible as was attempted by Hanzlik and Weidenthal[4, p. 190] to control conditions in two wounds so that the effect of the coagulant on one of them can be recognized or can be measured quantitatively.

Hemostatic serum requires an appreciable time for activation by mixing with the fresh blood, before it can exert its characteristic property of shortening coagulation time. Test-tube tests, the addition of the agent to blood in a tube, are therefore valueless since this coagulant is exibited in a stable form which requires activation by fresh blood before its peculiar properties become effective. The method of N.N.R.[5] and Howell's method[2] using plasma are of little value for any coagulant, but especially in comparison with the systemic administration of the substance.

The definite results obtainable by systemic administration point to intravenous administration as an exceptionally valuable method for purposes of standardization. In order to determine the potency of the agent, its power to decrease the coagulation time of the blood in the living body should always be determined. Such a test can be made an accurate measure of value by establishing a minimum standard. There has not been, heretofore, any systematic scheme for standardizing blood coagulants—a fact which accounts, not only for the various attempts to establish values, but also lack of uniformity in the products marketed.

It is not sufficient to find, for example, that a freshly prepared extract of blood platelets is an active coagulant and expect that such an extract will invariably be active and stable. There are too many opportunities for error in preparation or for loss of activity through bacterial or chemical action to assume an activity which it is possible to demonstrate.

There is a tendency to decry the biologic methods of assay on the ground that the action of a therapeutic agent on an animal is no evidence of similar activity on the human. While this argument is illogical in most cases, it can scarcely be even advanced in the case of a blood coagulant. If an agent will shorten the coagulation time of a dog's blood, the blood differing in no essential respect from human blood, even to the average coagulation time, there is every reason to expect a similar action in the human. Then further, if clinical tests show almost identical results with those obtained by using the dog as the test animal, every reasonable objection to the biologic test vanishes.

The method of procedure proposed for standardizing blood coagulants of this character by the biologic test is as follows: The dog is anesthetized for convenience of withdrawal of samples of blood and for injection of the coagulant. After complete anesthesia the normal coagulation time is determined. The most convenient and accurate method is to use a sample of about 3 c.c. of blood. A glass cannula is inserted into a carotid artery for obtaining samples of blood. At least three samples should be tested for the normal coagulation time before injecting a coagulant. The blood is drawn into a clean test tube and set in water at 38° to 40° C. It is examined at one-minute intervals to observe the first evidence of fibrin formation and the progress of coagulation.

The normal coagulation time for the test animal has been selected as that time when on inverting the tube no fluid can be observed. One must be careful that an apparently firm clot is not due to a skin over the surface below which the blood is fluid. It is also advisable to clean out the cannula carefully before drawing the second and subsequent samples of blood in order to prevent particles of clotted blood from getting into the test sample.

If the coagulation time is found to be uniform at 7 to 10 minutes, the coagulant can then be injected into the femoral vein. The first evidence of shortened coagulation time usually does not appear until one-half hour after administering the agent, and the maximum effect not before one and one-half hours. Between those periods a sample of blood should be tested every fifteen minutes. This sample is examined to observe the first evidence of fibrin formation, the progress of clotting, and the time when a solid clot is formed which can be shaken and inverted without showing more than a trace of fluid blood.

The different blood coagulants examined have given variable results.[6] Some have appeared to be inactive, while some have shown exceedingly high efficiency. Clinical or other corroborative tests being absent in some cases, one can say only that for shortening the coagulation time of normal blood a combination of the factors concerned in this process seems to be most efficient. While in many cases the coagulation time was shortened to one-sixth or one-eighth of the normal, the average value selected for purposes of comparison is a shortening of the coagulation time to one-third the normal for that animal, the amount injected being 2 c.c. to a 12 kg. dog.

The method here described is an elaboration of an experiment by Howell,[2] who tested a sample of cephalin by intravenous administration, finding a coagulation time shortened by one-third to one-half the normal for that animal.

Some experiments of Drinker and Drinker[7] might suggest that this method is not applicable because of the tendency of hemorrhages to be self-limiting. The experiments of Drinker and Drinker were carried out very differently, however, since approximately ten times as much blood in proportion to the size of the test animals was used in their experiments as is needed for this method of standardization.

That repeated withdrawals of relatively small quantities of blood do not affect the coagulation time materially has been demonstrated not only intentionally but also involuntarily as when testing a valueless product. From dogs of approximately 12 kilograms weight, 5 c.c. of blood can be drawn repeatedly at 15 minute intervals or less over a period of 1 to 2 hours with no other evidence of disturbance of coagulation time than a slight irregularity. Exceptions occur, however, but it is more common to find a dog which does not react to the standard dose of an active coagulant than to observe a shortened coagulation time with no coagulant injected.

Charts of tests are attached showing these phenomena. Being a physiologic test it is subject to the limitations of the test animal. Not every animal is adapted to this test. In the same way some frogs, rats, guinea-pigs, and dogs are necessarily rejected in other physiologic tests because of having exceptionally high or low resistance to the agent.

Animals have been rejected for testing purposes: (1) Because of having too short a normal coagulation time: it should be not less than 6 minutes. (2) Because of some unrecognized factors which influence the coagulation time before the agent is administered. A shortened coagulation time on the second sample of blood to one-third that of the first sample was once observed when not more than 10 c.c. had been withdrawn from a 12 kg. dog. (3) Because of failing to react to an active sample of a coagulant, whose potency had been demonstrated on two test animals.

Three articles have recently appeared on the testing of thromboplastic agents, two[4] of them as to potency and one[8] as to the danger connected with the use of such agents.

In the tests for potency, Hanzlik and Weidenthal[4, p. 206] were unfortunately deterred from applying the most reliable test for such agents, namely, the physiologic test by systemic administration. They incorrectly quote Howell as authority for the statement that hemorrhage itself tends to shorten the coagulation time of the blood. Drinker and Drinker[7] quoted by Howell had shown this to be true, but Hanzlik and Weidenthal failed to note that the shortened time in the coagulation after hemorrhage was where the hemorrhage is excessive, as,

for example, an approximate loss of 20 per cent of the blood at one time, at another 33 per cent, the normal coagulation time being shortened by one-sixth and one-eighth, respectively.

Hanzlik and Weidenthal[4, p. 205] in their experiments observed a shortening in the coagulation time from 9 minutes to 3/4 minute in one case and from 8 minutes to 1¾ minutes in another. No data are included to show the extent of the hemorrhage.

In my own experiments no such procedure was adopted and, except rarely, no such results have been observed. The total amount of blood drawn for observation need not exceed 5 per cent of the total blood, and it is drawn at any one time in an amount not to exceed 0.5 per cent of the total blood. The careful withdrawal of this quantity does not affect the coagulation time.

Tests of thromboplastic agents *in vitro* are always unreliable and not to be compared to the physiologic test. Activity *in vitro* is not sufficient evidence on which to base the conclusion that in the body a similar result would follow, because in the circulating blood conditions are radically different from those in a test tube.

This is well illustrated by Hanzlik and Weidenthal,[4] who obtained no satisfactory results from any thromboplastic agent in experiments on the living animal, although some had shown high efficiency when tested *in vitro*.

It should not be overlooked that with few exceptions the thromboplastic agents tested by Hanzlik and his associates have been submitted to the Council on Pharmacy and Chemistry and accepted. Failure, therefore, on the part of these agents to show their expected effects should first be considered from the point of view of the method by which it was attempted to demonstrate their activity.

Scarcely second to this, however, should be the question of whether the nature of the coagulant is such as to be generally applicable for influencing the coagulation time of the blood both in health and disease.

A well balanced agent containing the elements essential for blood coagulation under all conditions, and with an efficiency demonstrated on the living animal, gives an assurance

of value not to be derived from tests on serum or plasma in the test tube.

It is unfortunate that the term "Anaphylactoid Phenomena" should have been used in connection with this third investigation.[8] One might assume this expression to mean phenomena similar to anaphylaxis. "Anaphylaxis is a state of unusual or exaggerated susceptibility to a foreign protein which sometimes follows a primary injection of such protein." (Richtet, 1893.)

The investigators state that these pigs were unsensitized, and further show by the experiments that only one dose was injected into each.

This precludes anaphylaxis, and we are forced to conclude that nothing resembling an anaphylactic reaction occurred. This conclusion is verified also by the descriptions of the observed reactions, which in the case of Coagulen containing no protein or only traces were identical with those from others in which the protein precipitate was unquestionable.

Attention should also be called to the doses used. In the case of hemostatic serum the smallest dose per gm. used by Hanzlik and his coworkers[8, p. 239] was 15 times as large as the maximum dose calculated over a period of 24 hours for a human, while the largest dose used was 80 times as large. Hemostatic serum is recommended in doses of 1 to 2 c.c., to be repeated in extreme cases as often as once in 4 to 6 hours. The largest single dose used in clinical practice is 2 c.c.,* which, when calculated as Hanzlik did from the weight of a 60 kg. human, is 0.000033 c.c. per gm. The largest dose used for the pig was 0.016 c.c. per gm., or 500 times the maximum single dose recommended for the human. If there were any lurking danger of anaphylaxis from administration of hemostatic serum or danger of poisoning because of the preservative or any other poison contained in it, certainly a dose of 500 times the maximum single therapeutic dose should have killed the animal. But in their whole series[8] only one animal died within 36 hours. This death was from a brain extract. The others were all killed by a blow on the head, in most cases having shown no serious effects other than those to be ex-

*Note: In certain conditions, 5 c.c. in one dose is recommended.—Author.

pected after an intravenous injection. This speaks well for the safety of hemostatic agents in clinical practice.

Hanzlik and his associates in their conclusions[8, p. 241] fail to make it clear that any one factor was responsible for the observed effects. It is not necessarily the protein content, for one of the agents tested contained practically no protein. Neither is it the preservative, for this same agent contained no preservative but at the same time gave the most pronounced reactions described as anaphylactoid.

It is logical to conclude, therefore, that the method of administration rather than the character of the agent may be responsible for the disturbances noted.

Administered intravenously to guinea-pigs few substances fail to produce more or less serious disturbances in the circulation and respiration, such as were observed by Hanzlik and his associates. It is illogical, however, to apply the term "anaphylactoid" to these phenomena because this word calls to the mind of the physician a severe reaction and occasional death from administration of antitoxin to a sensitized patient.

SUMMARY.

Without attempting entirely to discredit attempts to estimate the efficiency of thromboplastic agents *in vitro* or by local application to bleeding wounds, a method of a different character is here proposed for the assay of such agents.

The method is logical because it duplicates one of the clinical methods of applying such an agent.

The results of the potency test are conclusive since the action of the agent on the test animal is identical with its action in clinical practice.

Careful observance of certain precautions which have been outlined, such as are necessary in all forms of physiologic testing, makes this method the most dependable for the quantitative determination of the efficiency of hemostatic agents which can be used intravenously.

PROTOCOLS OF TESTS.

In the following tables, the first column of figures refers to the time of observation of the sample of the blood.

At the head of the columns that follow is the actual time

when the sample of blood is drawn or a sample of coagulant is injected.

The figures below the time are symbols: 1, signifies beginning of fibrin formation; 2 and 3, progressive clotting; 4, solid clot when tube is inverted.

HEMOSTATIC SERUM No. 053129.

									Feb. 2, 1920.		
	9:35	9:43	9:48	9:57	10:05	10:23	10:45	11:02	11:14	11:27	11:29
1.	—	—	—	—	Inj. 2 c.c.	—	1	3	3	4	4
2.	—	—	1	—		1	1	4	3		
3.	1	3	2	1		2	2		4		
4.	2	4	3	2		4	3				
5.	3		4	4			4				
6.	4										

Shortened time from 5 minutes to 1 minute in 1¼ hours.

HEMOSTATIC SERUM No. 051196.

Minutes	9:56	10:08	10:20	10:22	11:21	11:31	11:44	11:52	11:57	2:15
1	—	—	—	Inj.	—	1	—	3	3	2
2	—	—	—	2 c.c.	—	2	1	4	4	—
3	—	—	—	of	1	3	2			1
4	—	—	—	sam-	2	3	4			2
5	1	—	1	ple	2	4				4
6	2	1	2		3					
7	2	2	2		4					
8	3	3	2							
9	3	3	2							
10	3	3	3							
11	4	3	3							
12		4	4							

Coagulation time reduced from 12 min. to 2 min. in 1½ hours.

TEST OF AN INACTIVE PRODUCT.

	1:25	1:33	1:35	2:00	2:16	2:30	2:49	3:00	3:02	3:23	3:51	4:01
1.	—	—	Injected 2 c.c.		—			Injected 2 c.c. more	—	—	—	—
2.	1	1		—	—	1	—		—	—	1	1
3.	2	1		—	1	2	2		1	1	2	2
4.	3	2		1	2	3	2		2	2	3	2
5.	3	3		2	2	3	2		3	3	4	3
6.	3	3		2	3	4	3		3	3		4
7.	4	3		3	3		4		4	4		
8.		4		4	4							

COAGULATION TIME.

Jan. 6th, 1920.

Dog weighing 8 kilo not dosed with any hemostatic.

Eleven samples of blood representing about 5 c.c. each time removed to observe any change in coagulation time because of repeated bleedings.

Min.	11:00	11:11	11:19	11:25	11:33	11:47	1:20	1:45	2:57	4:01	4:34
1.	—	—	—	—	—	—	—	—	1	—	1
2.	—	—	—	—	1	1	1	1	2	1	2
3.	1	1	1	1	3	2	2	2	3	2	3
4.	2	2	2	2	4	3	3	4	4	3	3
5.	3	3	4	3		4	4			4	4
6.	4	3		4							
7.		4									

An illustration of one form of failure which occasionally occurs in attempting to test a coagulating agent.

May 21, 1919.

Minutes	10:33	10:43	10:49	10:52	11:48	1:31	2:11	3:00
1.	—	1	1	4	2	1	1	2
2.	—	2	4		4	3	2	3
3.	1	3				4	3	4
4.	2	4					4	
6.	2							
7.	3							
8.	3							
9.	4							

No injection was made into this dog as the coagulation time would not remain uniform at any time.

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Reprint No. 253, 1920.

(Reprinted from the *Journal of the American Veterinary Medical Association,* Vol. LVII, N. S. Vol. 10, No. 4, July, 1920.)

STUDIES ON ANTHELMINTICS.

IX—Santonin.

By Maurice C. Hall, Ph. D., D.V.M.

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In previous publications the writer has pointed out that since santonin differs from the great majority of anthelmintics in that it is not a gastro-intestinal irritant, it is especially qualified for use where repeated doses of an anthelmintic are indicated. Moreover, in the amounts in which santonin is commonly employed, repeated doses are much more effective than single doses, even where the latter exceed the limits of the commonly used therapeutic dose. Of the worms in the dog, hookworms and whipworms are the ones which commonly require repeated doses for their removal. Of these, the hookworms are not amenable to treatment with santonin, a fact which is generally known and which the writer has confirmed experimentally. Whipworms, however, can be removed by santonin, and in the writer's opinion santonin finds its especial indication in the treatment of whipworm infestations. In an earlier paper (Hall, 1917) the writer has stated the case thus:

"To secure results from santonin it is necessary to repeat the dose a number of times. It may be given (for ascarids), 1 grain of santonin and 1 grain of calomel a day, as often as necessary, having due regard for its effect on the patient, and especially on the kidneys, and in some instances this method might be preferred to the administration of a single dose of oil of chenopodium, but for certainty of results and saving of time, chenopodium is the preferred treatment. The treatment just outlined, 1 grain of santonin and calomel daily over long periods, is a very effective treatment for the removal of whipworms, as only an occasional dose of anthelmintic enters the cecum, where these worms are lodged, and treatment must be repeated to insure removal of these worms. Santonin is of no value against hookworms, even in oft-repeated doses."

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*Resigned March 27, 1919.

The following experiments in administering santonin to dogs illustrate the points just mentioned.

In single dose:

Dog No. 178, weighing 7.75 kilos (17 pounds), was given santonin at the rate of half a grain per pound of live weight, or 8.5 grains. This is the dose rate used by some veterinarians and is very much higher than that given by Winslow (1 to 3 grains). The dog received an equal amount of calomel. This dog passed either 57 or 20 ascarids, probably 20 (the feces of this dog and another were confused when both animals escaped from their cages). On post-mortem examination this animal had 12 ascarids and 1 *Dipylidium*. This large dose of santonin was therefore 62.5 per cent (or 83 per cent) effective against ascarids and 0 per cent effective against *Dipylidium*.

In repeated doses:[1]

Dog No. 110, weighing 13.6 kilos, was given a grain of calomel and a grain of santonin daily for a total of 6 grains of each in 7 days. The dog passed no worms and was found to have 2 whipworms post-mortem. Six doses were insufficient in this case to insure entry of the drug into the cecum, and the treatment was 0 per cent effective against whipworm.

Dog No. 111, weighing 10 kilos, was given the same treatment and for the same length of time as the preceding dog, No. 110. This dog passed 33 ascarids in the 6 days following the first dose, and on post-mortem was found to have 1 ascarid and 1 whipworm. Six doses were insufficient to insure entry of the drug into the cecum or the removal of all ascarids present. The treatment was 97 per cent effective against ascarids and 0 per cent effective against whipworms.

Dog No. 108, weighing 9.5 kilos, was given the same treatment, 1 grain each of santonin and calomel daily, for a total of 12 grains in 14 days. On the second day after beginning treatment the dog passed the thick posterior portion of a whipworm and on the third day passed the thin anterior end which is habitually found sewed into the mucosa, showing that following the toxic effect on the worm peristalsis had apparently torn the free portion of the dead worm from the attached portion, the attached portion subsequently becoming released and passing out a day later. On post-mortem

[1] This set of protocols was published by Hall (1919).

the dog was found free from worms, the treatment being at an early stage 100 per cent effective against whipworms.

Dog No. 71, weighing 12 kilos, was given daily doses of 1 grain each of santonin and calomel, approximately 2 days out of every 3, for a total of 61 grains in 3 months. After 7 doses the dog passed 1 whipworm. Post-mortem the dog had 32 hookworms and 4 *Dipylidium.* The treatment was, therefore, 100 per cent effective against whipworms and 0 per cent effective against hookworms and *Dipylidium.* The fact that over a dram of santonin was without effect on hookworms or tapeworms is rather conclusive evidence as to its entire lack of efficacy against these worms.

Dog No. 120, weighing 13.5 kilos, was given 5 grains each of santonin and calomel daily for a total of 25 grains in 6 days. This dose invariably caused vomiting in the course of a half-hour, so the daily dose was cut to 2.5 to 3.5 grains and 9 such doses given in the next 10 days for a total of 25.5 grains. The dog was given a total of 50.5 grains in 16 days, an average of over 3 grains a day The third day after the first dose the dog passed 14 whipworms and was free from worms post-mortem. The treatment was therefore 100 per cent effective against whipworms.

Santonin combined with oil of chenopodium:

Dog No. 306, weighing 13.5 kilos, was given 1 dose of 3 grains each of santonin and calomel, and oil of chenopodium at the rate of 0.1 m. p. k. (mil per kilo). This treatment removed 43 ascarids and left 5 *Dipylidium.* Efficacy against ascarids, 100 per cent; against *Dipylidium,* 0 per cent.

Dog No. 308, weighing 14 kilos, was given 2 grains each of santonin and calomel, and oil of chenopodium at the rate of 0.05 m. p. k. The dog passed 1 ascarid, and on post-mortem was found to have 1 ascarid left. The treatment was, therefore, only 50 per cent effective against ascarids. This experiment does not indicate any synergistic action from the simultaneous use of santonin and chenopodium. Sollmann's (1918) tests on earthworms *in vitro* did not indicate that synergistic action is to be expected. As has been stated, santonin does not develop high efficacy in single doses, even in large doses, and gives its best results by what seems to be a cumulative action against such worms as ascarids. As regards chenopodium, the therapeutic dose against ascarids is 0.1 m. p. k.; the dose used here (0.05 m. p. k.) is commonly 100 per cent

effective, though not dependably so, as the dose of 0.1 m. p. k. may be said to be.

Dog No. 310, weighing 8 kilos, was given 1 grain each of santonin and calomel, and oil of chenopodium at the rate of 0.05 m. p. k. The dog passed 6 ascarids and had 28 *Dipylidium* post-mortem. The treatment was 100 per cent effective against ascarids and 0 per cent effective against *Dipylidium*.

Dog No. 13, weighing 9 kilos, was given 2 grains each of santonin and calomel, 25 minims of oil of chenopodium (almost 0.2 m. p. k.), and 0.1 grain of elaterin. The dog passed 2 ascarids and had 3 *Dipylidium* post-mortem. The treatment was 100 per cent effective against ascarids and 0 per cent effective against *Dipylidium*.

An examination of the foregoing protocols indicates that in single dose santonin fails to show a very high anthelmintic value against ascarids, even when used in doses of a half-grain per pound of live weight. The protocols show that in repeated doses it manifests what appears to be a cumulative action against ascarids, gradually clearing them out. Repeated daily doses of 1 grain each of santonin and calomel will ultimately clear out whipworms. This may not be accomplished in the course of a week in some cases, and it would perhaps be advisable to give this treatment for 1 week, suspend treatment for a week, and then repeat for a week. The assurance of a cure would have to be obtained from fecal examinations for eggs; generally speaking, the diagnosis of whipworm infestation would be made in the same way. The protocols suggest that a successful termination to treatment for whipworms by repeated doses might be hastened by giving larger doses of santonin daily. Whether one cared to give the larger doses would depend partly on his judgment as to whether it was safe. So far, all our experience with santonin has indicated that it is quite a safe drug for dogs in the doses commonly employed; we have yet to see a dog killed with the drug, and the protocols show that the doses employed are in some instances rather large when compared with those commonly advocated for dogs.

Winslow (1913) says: "While 5 to 6 grains induce symptoms of poisoning in dogs, ½ to 1 dram has often failed to produce a fatal result. . . . Santonin is very slowly absorbed from the intestines and is oxidized in the tissues and eliminated as oxysantonins."

The protocols show that santonin is entirely without value against hookworms and *Dipylidium*, the use of over a dram of santonin in 3 months having no effect on hookworms and *Dipylidium* in the case of dog No. 71, and the use of half a grain per pound having no effect on *Dipylidium* in the case of dog No. 178.

In passing, it may be noted that Hall and Foster (1918) found that santonin in the 1- to 3-grain doses was only 24 per cent effective against ascarids, the efficacy rising when two doses were given instead of one. They likewise found santonin, under these conditions, entirely ineffective against hookworms and tapeworms, and only 7 per cent effective against whipworms. These findings are in agreement with those published here.

In connection with the administration of large amounts of santonin, it is interesting to note that in the case of dog No. 71, which received a little over a dram in the course of 3 months, the animal's weight dropped from 12 kilos to 10 kilos in 1 month; to 9 kilos in 7 weeks; and rose slightly, to 9⅛ kilos, 1 week before the animal was killed. This dog's eyes were very luminous, and the effect was heightened by the fact that the hair came out over a fairly wide area around the eyes. There was also a pronounced loss of hair along the ventral surface of the neck and abdomen and in the axillary and inguinal regions. Sores formed around the nose. The dog was very active. In spite of the large amount of santonin administered, the digestive tract was normal except for a few small inflamed areas in the jejunum, the drug manifesting its customary lack of irritant qualities. Dog No. 120, which received 50.5 grains of santonin in 17 days, had a normal digestive tract.

Under the initials S. A. K. (1919), a writer has recently raised the question as to the advisability of using santonin in human cases where fever is present, as follows:

"I have met with . . . a variety of cases with high fever and history of vomiting or having passed a worm or two per rectum. I want to know whether santonin can be given when the fever is over 102 degrees. If not, what else should be tried? I used santonin in fever with fatal results. The fever does not subside for days together with any remedies. In such cases, knowing as I do that the cases are complicated with worms, I am at a loss to know how to give relief to my patients."

I have elsewhere expressed the opinion that febrile conditions

are contraindications to anthelmintic treatment. There might be cases where it would be advisable to use anthelmintic measures during the course of a febrile disease, but I doubt it. Febrile conditions indicate the presence of toxins, and the administration of additional toxic material in the shape of anthelmintics would commonly be unwarranted. As regards the effect of the santonin on the temperature of the patient, our experiments show the following results:

Dog 71 started with an initial temperature of 100.8° F.; after a slight rise, this began falling and went to 99.6° in a week; there followed a gradual and irregular rise, reaching a maximum of 102.4° in 3 weeks; following a gradual 4-day drop to a little over 100°, the temperature jumped to over 102° and gradually fell to a minimum of 99.1° 5 weeks after the beginning of treatment; thereafter the temperature maintained a range between 100° and 101° almost all the rest of the time.

The slight initial rise in temperature following administration of santonin is duplicated in the case of 4 out of 5 remaining dogs. Following this there is a drop and then the temperature fluctuates, but in these experiments the temperature rarely deviated from the normal range, and then the deviation was small as a rule. Santonin apparently has but little effect on the temperature of the normal animal. Dog No. 71 showed little deviation from the range of 99.5° to 102.2° given by Malkmus as the normal for dogs, and dog No. 112 did not even reach these limits of deviation; these were the dogs receiving over 60 and over 50 grains of santonin, respectively.

In passing, the following facts from the *Chemist and Druggist* are of interest:

Some twelve years ago Germany acquired a monopoly of the santonin industry in Turkestan, and Hamburg was a center of the distributing trade. During the war exportation from Russia has been in the hands of one firm. There is only one factory producing santonin, and that is near Tashkent in Russian Turkestan. It now develops that for several years past no wormseed has been collected and that for 15 months manufacture has practically ceased. This is attributed to an acute famine which has prevailed, lack of labor, and insufficient crops of wormseed. There has also been a shortage of hydrochloric acid, which is used in the process of manufacture.

These factors have caused a considerable shortage in santonin, and even after the war is finished and communications reëstablished, it will require one or two years before the Turkestan factory is able to resume its normal annual output of 8,000 to 10,000 kilos.

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Parke, Davis & Co.
Reprint No. 254, 1920.

(Reprinted from the *Journal of the American Pharmaceutical Association*, Vol. IX, No. 6, June, 1920.)

BIOLOGIC ASSAYING: ITS SCOPE AND LIMITATIONS.*

By Herbert C. Hamilton.

To many of you this subject of biologic standardization may seem hackneyed and time-worn. Among my earliest recollections in connection with this subject was a controversy between the representatives of two pharmaceutical manufacturing firms as to whether it is possible to make the test quantitative, neither party questioning its truly qualitative character when properly applied.

Now, however, the question seems to have advanced a point. It is apparently doubtful in some minds whether it is even qualitative. It was stated recently that "If you would know the effect of a drug on a human it must be tested on a human; this cannot be deduced with any degree of certainty by its action on one of the lower animals."

Is there any excuse for continuing an apparently profitless discussion? There is more than an excuse, there is a reason and a vital one. To each of us, either for himself or for some one near and dear to him, it is a vital question, since few of us are fortunate enough to escape the physician and the druggist.

If you respond that most of the drugs we use are standardized chemically or are so harmless that they need no standardization, it is really a strong point for biologic standardization, for why should any powerful agent be left to chance if a method can be applied by which a uniform product results?

Is there any less reason why the physician and the patient should be able to purchase standardized digitalis, ergot or antitoxin than for us to be able to buy standardized solutions of strychnine or morphine?

But some will say that standardization of digitalis does not

*Read before Scientific Section, A. Ph. A., City of Washington meeting, 1920.

insure potency when you buy it some months or years afterwards. But it does insure the marketing of a uniform product from a drug which is highly variable.

Digitalis grows under many varying conditions of climate, season and soil, sometimes cultivated, sometimes not. The time of gathering, the method and efficiency of the drying, the extraction, all may influence the activity of the final extract. Should this be left to chance if it is possible to make it a certainty?

But, you may reply by the question "How much certainty is there when the basis of the test is only that the drug will kill a dog, cat, pig, frog or goldfish?" That question, however, is really beside the point. The question of killing is unimportant; it is the amount that kills and the character of the death. If two tinctures of digitalis are tested on cats or frogs and one is found to kill with one-half the dose required for the other, which would the physician choose? Or, if two tinctures are tested on frogs and one stops the heart in systole while the other, although equally toxic, consistently leaves the heart in diastole, and when tested on the laid-bare heart does not slow the rhythm, one must conclude that there is little digitalis in the solution. The latter may contain some digitalis activity and is certainly toxic, but from causes other than the digitalis glucosides. This case is not a probable one, but it is always possible.

But you say again, what connection is there between the dose that will kill a frog and the therapeutic dose? None whatever! Neither is there any connection between the amount of acid used in titrating an alkaloid and the dose of the alkaloid in any particular case. The conditions are parallel, except that in the chemical assay it is possible to have a reagent of known strength or purity, while in the biologic assay the reagent—the animal—is a variable factor and must be checked up for its sensitiveness at time of assay.

A knowledge of pharmacology must precede biologic assaying. The drug must first be studied carefully on animals to determine in what respects it is most active if it has more than one typical effect. Among these effects one is to be selected as being typical and measurable, that is, showing degrees of activity dependent on the size of the dose.

But, you say, pharmacologists do not agree among themselves! They are not able to decide which is the most accurate test. That is unfortunately true. This, however, may not be a serious objection, for each investigator uses the method best adapted to the equipment of his laboratory and to his mental attitude.

It is not illogical at this point to note some of the different reactions which follow the administration of drugs.

Digitalis slows and strengthens the heart-beat and raises blood pressure by its action on the arterial walls. In toxic doses it causes arrhythmia, heart-block, and finally death. In frogs the heart stops in systole with a dose somewhat less than that which causes death. At death the heart is in systole, which is typical of the digitalis series of heart tonics. Ergot both raises and lowers blood pressure because it contains principles which have opposite effects. It causes stasis of blood in the vessels. It acts on the uterus muscle, causing contraction. Cannabis sativa causes typical intoxication in dogs with incoördination, one of the most characteristic features, with lowering of temperature and drowsiness.

It is evident from the above why pharmacologists differ in their opinions as to which effect is most typical or capable of most exact measurement.

But a still more serious objection has been voiced—that one cannot conclude from the action of a drug on animals what its action will be on man.

Partly true again. But can one be certain that any drug will invariably have the same effect on one human that it had on another? Answering the objection that the test and the clinical results are unlike is best done by illustration. Antitoxin for diphtheria neutralizes the toxins of the disease. That is practically its only function. It is assayed by neutralizing a toxin. The potency of the unneutralized toxin is determined on pigs; then its decreased potency is measured after partial neutralization, known amounts of the toxin being treated with different amounts of the antitoxin until its potency is destroyed.

Pituitary extract activity is measured by its constricting action on muscular tissue, the stronger the solution the greater the constricting action. It is measured on the uterus muscles

because it is used clinically to bring about contractions of this particular muscle. It is also measured on the muscles of the arteries because clinically it has application to overcome the collapse following operations. This seems to be directly dependent on the blood pressure.

Adrenalin and suprarenal extracts are not only used but also standardized as hemostatic agents. All through the list of the biologically tested drugs the same rule holds good—the method of testing runs closely parallel with some use of that drug in therapeutics. The only important difference is that while the biological assay is based on the same reaction that gives the drug its therapeutic value, the therapeutic dose is often small in proportion to the assay doses: the latter must produce an extreme effect observable in a short time, the therapeutic dose an almost intangible immediate effect.

The logic of this objection is at fault because of confusing a quantitative assay with a qualitative or pharmacologic study of a drug. It must not be overlooked that the assay process is not a qualitative study. The pharmacology of the drug must have been studied first just as qualitative precedes quantitative analysis.

About this time some one should say "But you pharmacologists are always saying how uncertain the biologic test is because the animal may not react properly just when you want it to!" True, alas, curses come home to roost! But in extenuation of this remark it must be observed that if the one animal, or set of animals, fails to react properly, we are apt to paraphrase the expression about the "perversity of inanimate things" and try again. It should not be overlooked that tests on the human sometimes do not permit of a second trial.

But how about the chemical test? Does it never go wrong?

Some years ago Haskell, examining some tinctures of aconite, all of which were adjusted to standard by chemical assay, found that they differed greatly in activity when tested on animals. One was 10 times as strong as the weakest one. But they looked alike and the alkaloidal content was almost identical in all of them. The alkaloid, while retaining its characteristic chemical properties, had evidently suffered some change which lowered its toxicity and also its therapeutic value. Clinical results are difficult to obtain with a drug of this character. Would you still say that one cannot deduce the physiologic action from the effect on the guinea-pig?

Has biologic assaying any limitations? Alas, yes! Not only do animals act erratically, sometimes responding to the drug with small doses and remaining immune to large ones in a truly human way, but we are at times obliged to ignore the most promising reaction of a drug because the effect is not measurable. For example, digitalis as a pressor agent, strophanthus, to measure its action on the heart directly, squill as an expectorant; even for the drugs commonly tested chemically we occasionally could use to advantage a measurable biologic test as a check but have no typical effect of this character.

Rusby says that many are skeptical as to whether the drug with the greatest power to kill a dog has the most value for curing a man. Thus baldly stated, the comment places biologic assay in an awkward light. Chemical tests may be more accurate, but it is no more difficult to show them to a disadvantage than to make a disparaging statement about the biologic tests.

Which test gives more assurance, to weigh or titrate the alkaloid or to test its activity on an animal in the same sense that it is active as a medicinal agent? The opium content of a solution is measured by the amount of sulphuric acid required to neutralize, while the identification of the active agent depends on the color developed when in contact with the concentrated acid.

To the uninitiated would it not be more reassuring if he were informed that a solution containing opium is identified by its sedative action on the dog and its potency measured by the amount required to put the dog to sleep in comparison with that of pure morphine or opium alkaloids?

The scope of biologic assaying is sharply defined with no function other than to measure the efficiency of remedial agents, which must otherwise remain of unknown potency.

The applicability of biologic tests, however, is unlimited if taken in the qualitative sense. By no other means can one more certainly identify the active agent of a drug or determine whether a drug has an active principle.

For example, in working with a solution containing strychnine it can readily be determined by tests on animals whether a certain chemical manipulation has affected the active agent materially.

Biologic tests have still another function which is twofold. For example, a statement was recently made that "The daily proof of the poor absorbability of strophanthus is had in the fact that

the dose given in the Pharmacopœia is the same as that for digitalis, though the Pharmacopœia requires that strophanthus shall be just one hundred times as active as digitalis."

This investigator failed to distinguish between two methods of administration which gives the complete explanation of this apparent phenomenon. Digitalis by mouth is less readily broken up in the stomach than is strophanthus, which must be administered in a relatively enormous dose in order that any may be absorbed before being acted on by the digestive ferments. When the active agents are intravenously administered, for example, into cats, dogs or pigs, comparable to that used in applying the frog assay method, or the cat method, the ratio of activities is not greatly different from the U. S. P. Standards.

This is a pharmacologic experiment which clearly demonstrates, first, the approximate correctness of the assay process; second, the proper method of using the different members of the digitalis series to get the full activity, promptly and without cumulative effects. Without animal experimentation neither of these facts could have been demonstrated.

Careful scrutiny of the ground which should be covered by the biologic assay processes and elimination of features which have no place there will do much to clear away certain logical criticisms.

Pharmacology is the study of the action of drugs by use of animals.

Biologic assaying is the use of animals as a means of standardizing drugs by comparing the action of two samples of the same drug, one of which is of known activity.

If we adhere strictly to this definition of the biologic assay it will eliminate most of the objections which have been raised.

There is no crying demand for information based on animal experimentation as to proper human dosage. Each individual is a separate problem for the physician and no elaborately worked-out dosage on the cat or other animal is of more than academic interest. But every one who is familiar with the variability of the crude drug and with the opportunities for errors and loss in preparing extracts knows that some method of standardization must be applied if possible. And what is more logical than a biologic test which not only measures effectiveness, although sometimes crudely, but also follows closely the therapeutic action of the drug?

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 255, 1920.

(Reprinted from the *Bulletin of Pharmacy*, Vol. XXXIV, No. 6, June, 1920.)

ADULTERATION OF AMERICAN CENTAURY AND MAIDENHAIR FERN.

BY OLIVER ATKINS FARWELL, DETROIT, MICH.

Deliberate adulteration of crude drugs is something seldom met with, but it has been my experience lately to detect instances that bear strong indications of such a practice.

Three bales of American centaury weighing 472 pounds contained just 113 pounds of drug true to name. There were two adulterants—*Rhexia virginica* Linn and *Stylosanthes biflora* (Linn) B. S. P. What more particularly points to a deliberate sophistication is the fact that all three species involved were definitely distinct, one from another; that is to say, they were not indiscriminately mixed, but each species occupied a distinct layer by itself.

In these bales the stylosanthes occupied the middle portion; the true American centaury was confined to the outermost layer so as to completely hide the adulterants, which were not at all discernible, in any of the bales, from the exterior; the rhexia occupied the space between the other two plants. It must have cost the ones who did the sophisticating considerable time and no little ingenuity to build up the bales after this fashion without getting any two of the species intermixed.

Neither of the adulterants bears sufficient resemblance to the American centaury to permit of either being intermixed without instant detection; hence the attempt at concealment.

In the case of the maidenhair fern the adulteration was of a somewhat different character but no less interesting.

A layer of drug alternated with a shovelful of coarse gravel in regular succession. There were five bags, the total weight of which was increased 50 pounds by having the gravel distributed in the manner outlined. Each shovelful of gravel was deposited as nearly

as possible in the middle of each layer of drug and amassed in a small pile so as to reduce the possibility of discovery to a minimum.

The insatiable greed, the everlasting desire to grab something for nothing, stops at no point and permeates every field of activity —such seems to be the conclusion that has to be drawn.

**Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 256, 1920.**

(Reprinted from the *Journal of the American Chemical Society*, Vol. XLII, No. 7, July, 1920.)

Derivatives of Trihalogen Tertiary-Butyl Alcohols. III. The Benzoic Acid Ester of Trichloro-Tertiary-Butyl Alcohol or Chloretone Benzoic Acid Ester.

By T. B. Aldrich.

(From the Medical Research Laboratories of Parke, Davis & Company, Detroit, Mich.)

Under the title, "Benzoyloxyisobuttersäure trichlorid," Willgerodt and Dürr[1] have described an oil which they obtained through the interaction of trichloro-tertiary-butyl alcohol on benzoyl chloride in molecular quantities. They state:

"On heating the mixture there is a rapid evolution of hydrochloric acid gas. The liquid, boiling at 270-290°, obtained by fractionation, is then dissolved in ether and shaken with an aqueous solution of sodium carbonate. After drying the ethereal solution, the ether is evaporated, and the yellowish oil remaining is distilled. The principal part of the ester distils between 275-280°, but on standing in the receptacle deposits crystals of benzoic acid which are removed by filtration. The oil thus obtained and further purified has finally a B. P. of 282° and is yellow in color. Chlorine determination: Theoretical 37.8%. Found 37.6%."

Having prepared a number of esters from trichloro- and tribromo-tertiary-butyl alcohols: acetyl chloretone,[2] acetyl brometone,[3] propionic and butyric esters of brometone,[4] and having obtained a *crystalline* benzoic ester of brometone (this work is unpublished) it occurred to me to repeat the work of Willgerodt and Dürr, especially since they reported their product as an oil, and compare it chemically, physically and pharmacologically with the other esters, and especially with the corresponding benzoic acid ester of tri-bromo-tertiary-butyl alcohol. The product I obtained by carrying out this synthesis differs so markedly from that obtained by the authors cited that I consider it imperative to publish the results of my investigation.

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[1]Willgerodt and Dürr, *J. prakt. Chem. N. F.*, 39, 286 (1889).
[2]This Journal, 37, 2720 (1915).
[3]*Ibid.*, 38, 2740 (1916).
[4]*Ibid.*, 40, 1948 (1918).

In carrying out the synthesis according to the very brief directions given at the beginning of this paper by Willgerodt and Dürr, the author finds that, during distillation under ordinary pressure, decomposition products are formed, consisting in part of benzoic acid, which may be recognized by its general appearance, solubility, odor, melting point, etc. Hydrogen chloride is also given off when the temperature is high. No doubt the product obtained by Willgerodt and Dürr is a mixture of benzoic acid, a small amount of the ester, the principal portion having been decomposed by the high temperature, and possibly some chloretone and other substances such as chlorine decomposition products. That benzoic acid is present is evidenced by the authors themselves, who state "the distillate deposits crystals of benzoic acid on standing." Indeed the method employed by the authors for purification would naturally lead to a mixture of various products rather than to a single product, since no attempt was made to remove the uncombined chloretone and benzoyl chloride that presumably did not enter into the reaction.

According to the method to be given shortly, any excess of chloretone or benzoyl chloride is removed by thorough washing and heating the product with alkali, which decomposes the ester very slowly, and then with water before any attempt is made to purify the ester.

That the chlorine determinations made by Willgerodt and Dürr agree so closely with the theoretical value, suggests the presence of bodies with high chlorine content mixed with other bodies containing little or no chlorine.

Although distillation *under ordinary pressure*, as practiced by Willgerodt and Dürr, leads to decomposition of the ester, distillation *under reduced pressure* can be carried out, as shown later, with very little, if any, decomposition.

EXPERIMENTAL.

Molecular quantities of chloretone, dehydrated over calcium chloride in a desiccator, and benzoyl chloride are heated on the steam bath for several hours, or until hydrogen chloride ceases to be given off to any extent. The reaction is practically ended in about 6 hours, although the product may be heated longer without injury.

As soon as the reaction is fairly complete, water is added, and the ester thereby thrown out in the form of an oil which solidifies when cooled in ice water. As the oil solidifies, it is best to agitate the contents of the flask to prevent the ester from forming a hard cake which is difficult to remove. When the ester has solidified, the supernatant liquid is decanted as completely as possible, an excess of caustic soda solution (5-10%) is added, and the vessel is heated on the steam bath for about half an hour. By this procedure, any chloretone or benzoyl chloride which remains will be decomposed, or rendered soluble in water. At the end of this time, the flask is cooled as before, and its contents poured into a mortar and ground to a fine powder. It is then transferred to a suction filter and washed a number of times with cold water. It dissolves readily, in alcohol, from which it is obtained in the form of white monoclinic crystals. If the reaction is carried out carefully the yield is excellent.

Chlorine determinations (Carius), carried out with a product which melted between 34-35° after several recrystallizations from moderately strong alcohol, gave the following results:

Subs., 0.2242, 0.2629: AgCl. 0.3426, 0.4027.
Calc. for $C_{11}H_{11}O_2Cl_3$: 37.83. Found: 37.80, 37.89.

The ester is readily soluble in strong alcohol, acetone, chloroform, ether, glacial acetic acid, benzene, etc., but very sparingly soluble in water. It may be recrystallized to advantage from alcohol, from which it may be precipitated by water.

Distillation.—(*a*) *Under normal pressure.* Twenty-three g. of the purified ester was distilled under ordinary pressure. Three fractions were obtained: (1) 170-200° (few cc.) (neglected) distillate colorless; (2) 200-250° (small portion); (3) 250-270° (greater portion). The residue was dark reddish in color, decomposition having taken place, since vapors were given off during the distillation. To the third fraction a little alcohol was added, and the solution was placed in a vacuum desiccator. Crystals formed after standing, m. p. about 112°. This would point possibly to benzoic acid, m. p. 120°.

Distillation.—(*b*) *Under reduced pressure.* Thirty-five g. of the purified ester was distilled under a pressure of 148 mm. Four fractions were obtained: (1) 190-220° (few cc.) slight coloration in distillation flask, distillate clear and colorless; (2) 220-225°

(about 5 cc.) color increasing in flask, fumes noted in receiver, distillate colorless; (3) 225-230° (about 5 cc.) color increasing slightly in flask, distillate colorless; (4) 230-235° (about 20 cc.) dark residue (small in amount) left in flask, distillate colorless.

All the fractions on cooling in ice water solidified to a mass of crystals, and, after recrystallization from alcohol, all gave the same m. p., 34.5 to 35°, the melting point of the original ester. There was a yield of 29 g. of purified product which shows very little decomposition. In this state the ester apparently boils under the above pressure without decomposition at from 220-235°. The volume of the several fractions was estimated.

The ester is very slowly volatile with steam, but does not decompose. It passes over as an oil which solidifies on cooling, especially when rubbed with a rod or when inoculated with a crystal of the substance.

Unlike chloretone, brometone, and the acetic esters, it is practically non-volatile in the air at ordinary temperature, as well as at incubator temperature (37°). The loss even after several days is very slight.

When boiled with water for 18 hours or with 10% sulfuric acid for several hours, very little decomposition occurred. The supernatant liquid gave a slight test for chloride, but in both instances practically all of the ester remained as an oil which solidified when cooled and melted between 34° and 35°.

However, when the ester is refluxed with 10% sodium hydroxide solution, it decomposes slowly, and chlorides may be recognized in the supernatant liquid. This resistance of the ester to the action of moderately strong alkali, as intimated previously, is utilized in its preparation to separate it from chloretone and benzoyl chloride, both of which are decomposed very quickly by this reagent, especially when heated with it. If the boiling with alkali is not continued too long, a portion of the ester remains undecomposed.

When *acetyl chloretone* is boiled with an excess of conc. nitric acid, saponification commences immediately and the presence of chloretone may be demonstrated in a few minutes if water is added; when acetyl, propionyl or butyryl brometone is treated in the same way saponification takes place also, but the brometone itself is decomposed to a certain extent as shown by the evolution of bromine vapors. When, however, the benzoic acid ester is treated similarly, the halogen complex apparently is not changed.

With water or 10% sulfuric acid in a sealed tube under pressure at a temperature of 160-170° for several hours the greater part of the ester is found undecomposed in the form of an oil, which, when cooled in ice water, solidifies, and after recrystallization, melts between 35° and 36°.

One fact stands out prominently: the benzoic ester is characterized by great relative stability, greater even than the other esters thus far studied. It also differs in toxicity, being 1/3 as toxic as the acetic ester of brometone, and about 1/10 as toxic as the corresponding acetic ester of chloretone. As far as observed, the benzoic ester does not produce convulsions, which is at variance with the observation of Wolffenstein, Lowey and Bachstex relative to analogous esters.[5] Perhaps owing to the insolubility of the ester, the properties referred to do not develop, but this is hardly to be expected since the other esters are also practically insoluble.

The following pharmacological facts relative to the ester were furnished by my associate, Mr. L. W. Rowe:

"When melted and mixed with olive oil and injected intraperitoneally into guinea pigs, its M. L. D. was found to be about 1.5 g. per kg. body weight.

"Experiments upon dogs indicate that the compound has very slight physiological action, even when given in very large doses. One dog when given 0.5 g. per kg. in capsule per stomach showed slight muscular incoördination 1½ hours later. No other effect.

"Another dog given twice the above dose (one g. per kg.) in the same way exhibited slight incoördination and uneasiness. This dog was killed 4 days later, and an examination of the stomach showed that no irritating action of a serious nature had taken place.

"A dog was given one g. per kg. by intraperitoneal injection. A slight sedative effect was observed in about 20 minutes. Marked diuresis and purgation resulted in about 30 minutes after injection. Slight nausea was observed about 2 hours after injection. Dog was very sick and died 20 hours after being dosed. Autopsy showed marked inflammation of the intestines and bladder, showing that the irritation caused by the material injected must have been largely responsible for the purgation and urination.

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[5]Wolffenstein, Lowey and Bachstex, *Ber.*, 48, 2035-43 (1916).

"Five g. of the melted sample was injected subcutaneously into a dog. No general effect was observed. Several days later the skin sloughed off at the site of injection, showing that the material was very irritating and was not properly absorbed.

"That irritation is produced when the ester is injected intraperitoneally or subcutaneously is not necessarily indicative of irritant properties inherent in the substance itself; but is probably, in part at least, caused by its non-absorption, just as with any other similarly introduced foreign material.

"The insolubility of the compound as well as the fact that it is apparently not broken up in the body into soluble constituents renders it difficult to study its pharmacological action."

SUMMARY.

The benzoyl ester of chloretone, $C_6H_5CO.OC\text{-}C_3H_6Cl_3$, is prepared by heating molecular quantities of benzoyl chloride and anhydrous chloretone on the steam bath until hydrogen chloride ceases to be given off. The ester is a solid which melts between 34-35° and not an oil as claimed by Willgerodt and Dürr, and distills under reduced pressure without decomposition. It is not readily saponified, and in this respect is much more stable than the other esters previously studied. Boiling with conc. nitric acid does not decompose it as is the case with the aliphatic esters of both chloretone and brometone. Pharmacological tests would indicate that it possesses less hypnotic or anesthetic properties and is less toxic than the esters studied thus far.

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 257, 1920.

(Reprinted from *The Journal of Biological Chemistry*, Vol. XLIII, No. 1, 1920.)

WATER-SOLUBLE VITAMINES.

I. Are the Antineuritic and the Growth-Promoting Water-Soluble B. Vitamins the Same?

By A. D. Emmett and G. O. Luros.

(From the Medical Research Laboratories of Parke, Davis & Company, Detroit, Mich.)

The object of the present paper is to present data which we believe indicate that a more definite distinction should be made, for the present at least, between the antineuritic and certain of the other water-soluble vitamins, particularly those that relate to the growth stimuli.

Since making our preliminary reports (1, 2) on this subject, Mitchell (3) has presented an excellent review of the literature, and we will limit ourselves to a brief summary of the published work that bears directly upon the particular phase of the problem as we have approached it; namely, the stability of the water-soluble vitamins to heat.

We have, therefore, made a compilation of the available data on the basis of the particular biological test for which the trials were planned with only a secondary consideration of the descriptive names associated with the vitamin employed.

In comparing the data in Tables I and II, it should be borne in mind, as Chick and Hume (4) have brought out, that the amount of ration consumed may carry an excess of the vitamin beyond the minimum requirements, and, if so, a partial destruction of the vitamin by heat, alkali, or other causes might not necessarily become evident in the biological tests.

Comparing the results in the two tables, they tend to indicate that the antineuritic and the growth-promoting vitamins, as measured by polyneuritic pigeons and young rats respectively,

TABLE I.

*Polyneuritis (Antineuritic Vitamine).**

Series.	Temperature and time of heating.	Destruction.	Reference.
Section I. Fowl: pigeon, chicken, and duck.			
A	100°C., moist heat.		
1a†	Egg yolk, 4 min.	None apparent.	Cooper (5).
2a	" " in presence of dilute alkali.	Total.	Steenbock (6).
3b†	Buffalo meat, several days.	"	Grijns (7).
4b	Beef, 30 min.	None apparent.	Holst (8).
5a	Yeast extract, 1 hr.	Slight.	Chick and Hume (9).
6a	Wheat embryo, 1 hr.	Very slight.	Chick and Hume (9, 10).
7b	Unmilled rice, 3 hrs.	None apparent.	Eijkman (11).
B	110°, autoclave, 30 min.		
8b	Beef.	Appreciable.	Holst (8).
C	113°, autoclave, 1 hr.		
9a	Wheat embryo (102–107° for 40 min.).‡	Slight.	Chick and Hume (10).
D	115°, autoclave, 2 hrs.		
10b	Unmilled rice, millet, oats, rye, barley.	Total.	Eijkman (11).
E	120°, 15 pounds pressure, 30 min.		
11b	Beef, eggs.	Marked.	Vedder (12).
12b	Dried peas, unhulled barley.	None apparent.	Holst (8).
F	120°, 15 pounds pressure, 1 hr.		
13b	Beef.	Total.	"
G	120°, 15 pounds pressure, $1\frac{1}{2}$ hrs.		
14b	Unmilled rice.	"	Weil, Mouriquand, and Michel (13).
15b	Barley.	"	Weil and Mouriquand (14).

TABLE I—*Concluded.*

Series	Temperature and time of heating.	Destruction	Reference
Section I. Fowl: pigeon, chicken, and duck.—*Concluded.*			
H	120°, 15 pounds pressure, 2 hrs.		
16b	Unmilled rice, Katjidgo beans, buffalo meat.	Total	Grijns (7),
17b	Horse meat.	None apparent.	Eijkman (11).
18b	Rye, unmilled rice, millet, oats, barley	Total.	Holst (8).
I	122°, autoclave, 1 hr.		
19a	Yeast extract and wheat embryo (110–117° for 40 min.).‡	Appreciable.	Chick and Hume (9, 10).
J	122°, autoclave, 2½ hrs.		
20a	Yeast extract and wheat embryo (118–124° for 2 hrs.).‡	Very marked.	" "
K	125°, autoclave, 2 hrs.		
21b	Unmilled rice and millet.	Total	Eijkman (11).
L	135°, autoclave, 2 hrs.		
22b	Unmilled rice, rye, millet, oats, barley	"	Holst (8).
Section II. Dogs.			
M	120–130°, autoclave, 1 to 3 hrs.		
23b	Horse meat.	Total.	Schaumann (15).
24b	Lean beef in presence of 10 per cent Na_2CO_3.	"	Voegtlin and Lake (16).
Section III. Cats.			
N	120°, 15 pounds pressure, 3 hrs.		
25b	Lean beef	Appreciable	Voegtlin and Lake (16).
26b	" " in presence of 10 per cent Na_2CO_3	Total	" "

* Designated by some as water-soluble B, growth-promoting factor, neuritic-preventing vitamine, and antiberi-beri vitamine.

† a, tested curatively by giving it to polyneuritic fowl, b, tested prophylactically by feeding normal fowl.

‡ Temperature of the substance itself and length of time it remained at this point

TABLE II.

Growth-Promoting Vitamine (Water-Soluble B).*

Series.	Temperature and time of heating.	Destruction.	Reference.
	Section I. Rats.		
A	90–100°C., dry heat, several hrs.		
1	Liver, heart, kidney, brain.	None apparent.	Osborne and Mendel (17).
B	100°, moist heat.		
2	Protein-free milk, 2 min.	None apparent.	Osborne amd Mendel (18).
3	Milk whey, 6 hrs.	" "	McCollum and Davis (19).
4	Extract yeast, 30 min.	" "	Drummond (20).
5	Extract wheat embryo, in presence of 0.28 per cent NaOH, 1 hr.	Total.	McCollum and Simmonds (21).
6	Extract yeast, in presence of 5 per cent NaOH, 5 hrs.	Marked.	Drummond (20).
7	Soy beans, navy beans, cabbage, 40 to 120 min.	None apparent.	Daniels and McClurg (22).
8	Soy beans (120 min.), navy beans (90 min.), cabbage (45 min.), in presence of 5 per cent $NaHCO_3$.	" "	" "
9	Carrots.†	" "	Denton and Kohman (23).
10	Yeast, 0.1 N NaOH for 21.5 hrs. in cold. 2 hrs. heating.	" "	Osborne, Wakeman, and Ferry (24).
C	105°, dry heat, several hrs.		
11	Meat powder (lean beef).	" "	Osborne and Mendel (25).
12	Beef extract.	" "	" "
13	Compressed yeast.	" "	Hawk, Fishback, and Bergeim (26).

* Designated by some as water-soluble B, antineuritic vitamine, and water-soluble growth-promoting accessory factor.

† Placed in cans, then immersed in water, and heated at 100° for 2 hrs.

TABLE II—*Continued.*

Series.	Temperature and time of heating.	Destruction.	Reference.
	Section I. Rats—*Continued.*		
D	120°, 15 pounds pressure, 20 min.		
14	Navy beans, cabbage.	None apparent.	Daniels and McClurg (22).
15	Extract, navy beans.	" "	" "
E	120°, 15 pounds pressure, 30 min.		
16	Extract yeast.	Marked.	Drummond (20).
17	Soy bean flour.	None apparent.	Cohen and Mendel (27).
18	Extract, navy and soy beans, in presence of 0.1 N NaOH.	" "	Daniels and McClurg (22).
F	120°, 15 pounds pressure, 40 min.		
19	Navy beans.	None apparent.	McCollum and Simmonds (21).
20	Soy beans.	" "	Daniels and McClurg (22).
21	Extract, soy beans.	" "	" "
G	120°, 15 pounds pressure, 1 hr.		
22	Wheat embryo, milk whey.	" "	McCollum and Davis (19).
23	Extract, navy bean, in presence of 0.1 N NaOH.	" "	Daniels and McClurg (22).
H	120°, 15 pounds pressure, 1½ hrs.		
24	Navy bean.	" "	McCollum, Simmonds, and Pitz (28).

TABLE II—*Concluded.*

Series.	Temperature and time of heating.	Destruction.	Reference.
Section I. Rats—*Concluded.*			
I 25	120°, 15 pounds pressure, 3 hrs. Lean beef, in presence of 10 per cent Na_2CO_3	None apparent.	Voegtlin and Lake (16).
Section II. Yeast cell.			
J 26	120°, 15 pounds pressure, 30 min. Yeast extract.	Slight.	Williams (29).

were fairly stable at temperatures around 100-105° C. At higher temperatures, the antineuritic vitamin seemed to be less stable to heat and alkali than the rat growth-promoting vitamin, as shown in the case of wheat embryo (Table I, Series I, and Table II, Series G) and in lean beef (Table I, Series M and N, and Table II, Series I). The fact that no data were given as to the amount of food consumed by the rats, dogs, and cats in Voegtlin and Lake's work (16) is unfortunate as one might then have been able to evaluate their data from the standpoints under consideration here.

EXPERIMENTAL.

The plan of the series of trials reported was to use the *same* source of water-soluble vitamins for the studies on poylneuritis in pigeons and the rate of growth in young rats.

The basal food employed was unmilled rice. This furnished the only source of the water-soluble antineuritic and growth-promoting vitamins. In the case of the pigeons, the unground rice constituted the sole food. Gravel was offered once a week. With the rats, the ground rice was so supplemented with lactalbumin, salt mixture (21), butter fat, and lard that it formed a balanced ration for growth. The fundamental difference in the diets of the respective groups of pigeons and rats was in the use of unmilled rice that was unheated for the controls and of rices that were heated at different temperatures.

The heating of the rice was carried out as follows: in the air oven at 120° C. for 2 hours, after the temperature reached this

point; and in the autoclave at 120° and 15 pounds pressure for 1, 2, and 6 hours, respectively—making in all four different samples. In some of the trials the pigeons were allowed to eat their food at will, others were force-fed as soon as they began to refuse their food. The pigeons were put in cages in groups of seven each. Each rat was kept in a separate compartment. The food intake of the rats was determined directly, that for the pigeons had to be calculated from the total consumption of rice for the entire group.

DISCUSSION.

Part I. Influence of Degree of Heating on the Antineuritic Vitamin, Using Pigeons.

The results presented in Charts 1 and 2 show the relative rate at which pigeons come down with polyneuritis when fed *ad libitum* unheated and heated unmilled rice. From these it is evident that some decided change took place in the rices that were subjected to the longer periods of heating. Naturally the first question that arises is in regard to the amount of the food consumed by the different lots. A fair idea of this is given in Table III.

The values are somewhat approximate due to the fact that the pigeons were fed in groups and also that they scattered some food. The refused food in the cups and on the metal floor of the cages was collected and subtracted from the total amounts offered. The results indicate that the loss in weight varied inversely with the amount of rice eaten.

TABLE III.

Food Consumed by Pigeons Fed ad Libitum.

(Gm. of food per day per 100 gm. of body weight.)

Period.	Unheated rice.	Rice, 1 hr. in autoclave at 120°.	Rice, 2 hrs. in autoclave at 120°.	Rice, 6 hrs. in autoclave at 120°.	Rice, 2 hrs. dry heat at 120°.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
1st week..........	7.6	8.1	8.0	7.2	8.8
2nd "	8.0	8.6	6.3	5.8	6.3
4th "	8.2	9.0	4.9	4.8	6.5
Average........	8.0	8.6	6.4	5.9	6.7

The fact that the food intake varied in this manner suggested that two or three factors might be involved in the causation of the onset of polyneuritis. The excessive heating might have destroyed the antineuritic vitamin; it might have produced certain toxic substances which prevented the vitamin from being potent; or it might have altered the physical condition of the rice, making it harder and less easily digestible.

In view of these possibilities, it was decided to force-feed pigeons and compare the effects produced with those fed *ad libitum*. These curves are presented in Chart 3. In the main the results obtained were identical with what was found when the pigeons were fed *ad libitum*.

The question of the formation of toxic substances is still unanswered. In view of this, it was decided to carry out a series of experiments by force-feeding pigeons on unheated *milled* rice and then treating them with small quantities of an extract of autolyzed yeast which had been heated in the same manner as the natural or unmilled rices in Chart 3. In this way, it would be possible to eliminate any resultant conditions produced in the unmilled rice during the heating, such as undue hardness, peculiar aroma, etc. Since the amount of the vitamin extract given in this treatment was relatively small, being 0.19 gm. daily, it would seem that the toxic effect, if any, that could have been caused by adding this material to the rice diet was too insignificant to be considered seriously. The results are given in the first half of Chart 9.

In order to test this point of toxicity further, curative trials were made with extracts containing the antineuritic vitamin both before and after heating. Besides, vitamin extracts were treated with fullers' earth according to the method of Seidell (30) and the activated silicate was used. The results are presented in Table IV. These results together with those in Chart 9 show conclusively, even if reasonable allowances are made for the indefiniteness that sometimes appears in cases of polyneuritic pigeons, that toxicity was apparently a minor factor and that the antineuritic vitamin was totally destroyed by heating for 2 and 6 hours in the autoclave at 120° and 15 pounds pressure.

TABLE IV.

Treatment of Typical Polyneuritic Pigeons with Heated and Unheated Vitamin Preparations.

Bird No.	Treatment.	Response to treatment.
331	0.3 gm. unheated yeast extract.	Good recovery.
326	0.3 " " " "	" "
333	0.1 " " " "	Improved.
300	0.5 " 6 hrs. autoclaved yeast extract in a.m.	No better. Given a second treatment in p.m.
	0.5 " 6 hrs. autoclaved yeast extract in p.m.	Found dead next morning.
293	0.5 " 6 hrs. autoclaved yeast extract in a.m.	No better. Given a second treatment in p.m.
	0.5 " 2 hrs. autoclaved yeast extract in p.m.	Found dead next morning.
303	0.5 " 6 hrs. autoclaved yeast extract.	" " " "
394	0.5 " 2 hrs. autoclaved yeast extract.	" " " "
332	0.5 " 2 hrs. autoclaved yeast extract.	" " " "
405	0.5 " 2 hrs. yeast extract, 1st day.	No better. Treated following morning. Good recovery.
	0.5 " unheated yeast extract, 2nd day.	
414	0.6 " unheated activated protein-free milk.	Very good recovery.
436	0.6 " unheated activated protein-free milk.	" " "
433	0.6 " activated 6 hrs. autoclaved yeast extract.	No better. Treated second day.
	0.6 " activated unheated yeast extract, 2nd day.	Very good recovery.
422	0.6 " activated unheated yeast extract.	Good recovery.
404	0.6 " 6 hrs. autoclaved activated protein-free milk.	Found dead next morning.
432	0.6 " 6 hrs. autoclaved activated protein-free milk, 1st day.	No better. Treated second day.
	0.6 " unheated activated protein-free milk, 2nd day.	Very good recovery.

Part II. Influence of Degree of Heating on the Water-Soluble B Growth-Promoting Vitamin, in Rats.

Four groups of young rats were so fed that a comparison of the rate of growth of each would indicate the relative degree of destruction of this water-soluble vitamin by heating the rice.

The ration for the control group was made up as follows:

		Per Cent
Period 1.	Unmilled rice, unheated	64.0
	Lactalbumin	6.0
	Butter fat	18.0
	Lard	10 0
	Salt mixture	2.0
Period 2.	Unmilled rice, unheated	89.7
	Lactalbumin	3.3
	Butter fat	5.0
	Salt mixture	2.0

Equivalent percentages of rice were used throughout, calculated on the dry basis, and then the necessary amount of water was added to bring all the rations to the same moisture content. We recognize that this method of adjusting the rations did not necessarily allow for the variations in palatability that might have been brought about during the heating of the rices. The food intake of each rat should, however, assist in interpreting the results from this standpoint.

The data are presented in Charts 4, 5, 6, and 7. When the individuals in the groups are compared, the greatest variation is found in the case of the 6 hours autoclaved rice (Chart 7).

In Chart 8 the average results for each group are given. These average group curves indicate that the heating processes apparently had some detrimental effect during the 6 hours in the autoclave at 120° and but very slight, if any, effect during 2 hours at 120° either in the oven or autoclave. A study of these data in connection with the food intake and the percentage gain per gm. of food consumed (Tables V and VI) will assist further in evaluating the rations.

In Table V, the amount of food consumed per day per rat is given. The differences in food intake between Groups 40, 50, and 60 were no greater than the differences between the values for the individual rats in the respective groups. In the case of Group 70, however, the rats ate less of the ration, except Rat 75, which consumed as much as some of the animals in the other

groups. The gains in weight of this rat compared favorably with those in the other groups having an equivalent food intake.

If the growth-promoting water soluble vitamin had been destroyed, the rate of growth for Rat 75 would have been much less. We are confronted, therefore, with the question of the

TABLE V.

Food Consumed.

Group No.	Ration and rat No.	Food consumed per day per rat. Rat 1.	Rat 2.	Rat 3.	Rat 4.	Group average.
	Period 1 (75 days).					
		gm.	*gm.*	*gm.*	*gm.*	*gm.*
40	Unheated rice. Rats 41, 42, 43.	5.9	5.6	4.2		5.2
50	2 hrs. dry heat 120°. Rats 52, 54, 56.	4.9	6.5	5.9		5.8
60	2 hrs. autoclave 120°. Rats 62, 63, 64, 66.	5.6	4.5	4.2	5.2	4.9
70	6 hrs. autoclave 120°. Rats 71, 72, 73, 75.	3.3	2.6	3.1	4.3	3.3
	Period 2 (50 days).					
40	Unheated rice. Rats 41, 42, 43.	10.8	10.1	11.5		10.8
50	2 hrs. dry heat 120°. Rats 52, 54, 56.	8.2	10.1	11.3		9.9
60	2 hrs. autoclave 120°. Rats 62, 63, 64, 66.	7.0	8.1	7.8	9.0	8.0
70	6 hrs. autoclave 120°. Rats 71, 72, 73, 75.	4.7	3.3	3.6	7.5	4.8
	Periods 1 and 2 (125 days).					
40	Unheated rice. Rats 41, 42, 43.	7.8	7.4	7.1		7.4
50	2 hrs. dry heat 120°. Rats 52, 54, 56.	6.2	7.9	8.0		7.4
60	2 hrs. autoclave 120°. Rats 62, 63, 64, 66.	6.1	5.9	5.6	6.8	6.1
70	6 hrs. autoclave 120°. Rats 71, 72, 73, 75.	3.9	3.2	3.7	5.8	4.1
	Period 3 (21 days).					
70	Unheated rice (same as No. 40). Rats 71, 72, 73, 75.	9.0	5.5	7.7	9.7	8.0

TABLE VI

Gain per Gm. of Food Consumed.

Group No.	Ration and rat No.	Gain per gm. of food. Rat 1.	Rat 2.	Rat 3.	Rat 4.	Group average.
	Period 1 (75 days).					
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
40	Unheated rice. Rats 41, 42, 43.	20.3	20.0	17.5		19.3
50	2 hrs. dry heat 120°. Rats 52, 54, 56.	20.9	17.7	22.2		20.3
60	2 hrs. autoclave 120°. Rats 62, 63, 64, 66.	18.2	20.0	14.5	16.1	17.2
70	6 hrs. autoclave 120°. Rats 71, 72, 73, 75.	15.1	(6.9)	13.0	16.6	14.9
	Period 2 (50 days).					
40	Unheated rice. Rats 41, 42, 43.	8.8	7.7	11.1		9.2
50	2 hrs. dry heat 120°. Rats 52, 54, 56.	8.3	4.0	11.4		7.9
60	2 hrs. autoclave 120°. Rats 62, 63, 64, 66.	6.7	7.1	12.6	12.7	9.8
70	6 hrs. autoclave 120°. Rats 71, 72, 73, 75.	10.6	(3.3)	3.5	11.5	8.5
	Periods 1 and 2 (125 days).					
40	Unheated rice. Rats 41, 42, 43.	14.5	13.8	14.3		14.2
50	2 hrs. dry heat 120°. Rats 52, 54, 56.	14.6	10.8	16.8		14.1
60	2 hrs. autoclave 120°. Rats 62, 63, 64, 66.	12.4	13.5	13.5	14.4	13.4
70	6 hrs. autoclave 120°. Rats 71, 72, 73, 75.	12.5	(5.1)	8.2	14.0	11.6
	Period 3 (21 days).					
70	Unheated rice (same as No. 40). Rats 71, 72, 73, 75.	19.0	(19.8)	18.0	24.1	20.4

palatability of the ration and as to what the other rats in the group would have gained if they had eaten as much food. This point is further evidenced in Period 3, where all the rats ate more and made better gains than in Periods 1 and 2. Osborne and Mendel (31) found similar conditions in palatability in the case of soy bean.

When the rations (Table VI) on the basis of the gain made per gram of food consumed are compared, it is evident that the diets for Groups 40, 50, and 60 were all practically of equal value. The data for Group 70, Period 1, compare favorably with the lower values for these groups. In Period 2, two of the rats did very well while the other two did very poorly. For the entire time, Rats 71 and 75 were able to utilize their food to as much advantage as the rats in the other three groups, showing that this ration was as economical as the others.

It is evident from Period 3 that all the rats in this group were capable of making good gains. Therefore, if their food consumption in Periods 1 and 2 had been equal to that of the rats in Group 40 and they had maintained the same degree of utilization of their food that they did in the test trial, the gains would have been larger than any of the other groups.

As another means of determining the effect of heat on the water-soluble growth-promoting vitamin, some rats were placed on a ration that lacked this vitamin and when they showed definite signs of retardation in growth 1 per cent of an extract of brewer's yeast, after being heated in the autoclave for 6 hours at 120°, was added. The diet was made up as follows:

	Per Cent
Lactalbumin	11.3
Starch	32.7
Lactose (purified)	24.6
Salt mixture	3.4
Butter fat	13.0
Lard	15.0

The curves are given in the second half of Chart 9.

SUMMARY.

In the foregoing discussion we have shown by comparing the antineuritic (pigeon) and the water-soluble B growth-promoting (rat) vitamins that in the former case the vitamin was altered by heating the food or extracts containing it to certain temperatures, while the other vitamin, obtained from the same food and extracts, apparently remained potent under the same conditions of heating. This is perhaps illustrated most definitely in Chart 9 where pigeons came down with polyneuritis on the one hand and young rats grew on the other.

The authors would call attention to the fact that the minimum vitamin requirement of the rats may have been lower per gram of body weight than that of the pigeons. This latter point was intended to be covered in part when the amount of unmilled rice in the rat rations was increased from 64.0 to 89.7 per cent. For, first, if the heated rices were toxic the rate of growth would be definitely lowered by feeding larger amounts of rice, and, second, if the supply of the growth-promoting vitamin was sufficiently low in the 64.0 per cent ration a slight or partial destruction of the vitamin by heat would be apparent. And upon increasing the amount of rice, the quality of vitamin would be increased and there would then be an added increment of growth.

The fact that the direction of the curves was not altered to any appreciable extent when this change was made suggests that the heated rices were not toxic, and, since the amount of water-soluble growth-promoting vitamin in the 64.0 per cent ration was apparently above the minimum requirements, these data are none too definite as to a partial destruction of the vitamin. However, it would appear that if the antineuritic vitamin was the same as the water-soluble B, in the treatment of pigeons with increasing doses of heated extracts and in the force-feeding of them with the heated rices there should have been some response if the vitamin was but slightly or partially destroyed. In order to answer this question definitely, it will be necessary to measure quantitatively the amount of vitamin consumed.

CONCLUSIONS.

1. The antineuritic vitamin (pigeons) in unmilled rice is stable to heat at 120° C. and 15 pounds pressure for 1 hour. It is partially altered by heating in the air oven at 120° for 2 hours, and totally destroyed at 120° and 15 pounds pressure in 2 and 6 hours. The vitamin in extracts is more easily altered by heat.

2. The water-soluble B vitamin (rats) in unmilled rice appears to be stable to heat at these same temperatures, that is, it is not distinctly or totally broken down. Whether this vitamin was slightly destroyed could not be definitely ascertained due to the lack of quantitative methods.

3. These findings suggest tentatively, at least, that the antineuritic (pigeons) and the water-soluble B (rats) vitamins are

not the same, and that it would be better to consider them as being different until there is further proof to the contrary.

The authors acknowledge their appreciation of the assistance of Miss Marguerite Sturtevant and Mr. Charles Hunter, in carrying out some of the details of the investigation.

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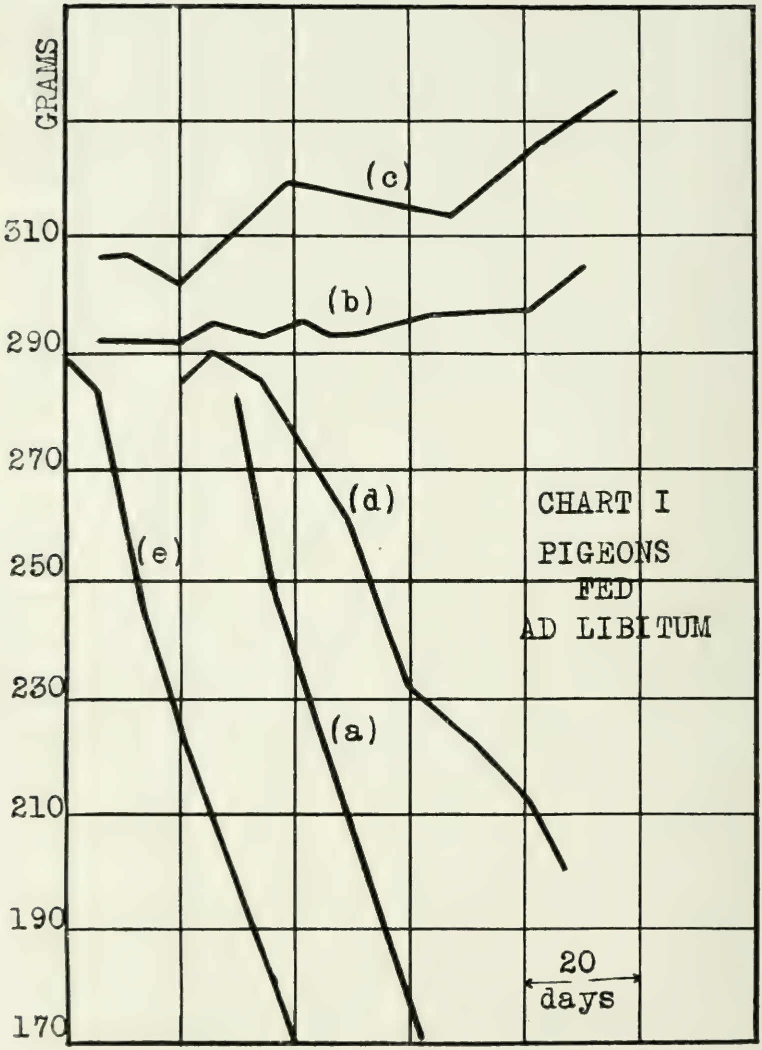

CHART 1. Represents the average body weight of the different groups that were fed *ad libitum*. Curve (a) for the milled rice; (b) for the unheated unmilled rice; (c) for the unmilled rice heated in the autoclave at 120° C. for 1 hr.; and (d) and (e) for the 2 and 6 hrs. autoclaved rices, respectively.

The heating for 1 hr. in the autoclave (Curve (c)) had no detrimental effect on the antineuritic vitamin. In the case of the 2 and 6 hrs. autoclaved rices, the losses in weight were very marked, beirg practically the same as for the milled rice (Curve (a)).

The pigeons on the 6 hrs. heated rice refused their feed and regurgitated part of it sooner than the ones that were fed on the 2 hrs. autoclaved rice. The general symptom complex of these two groups of pigeons was very similar to that observed in the feeding of milled rice.

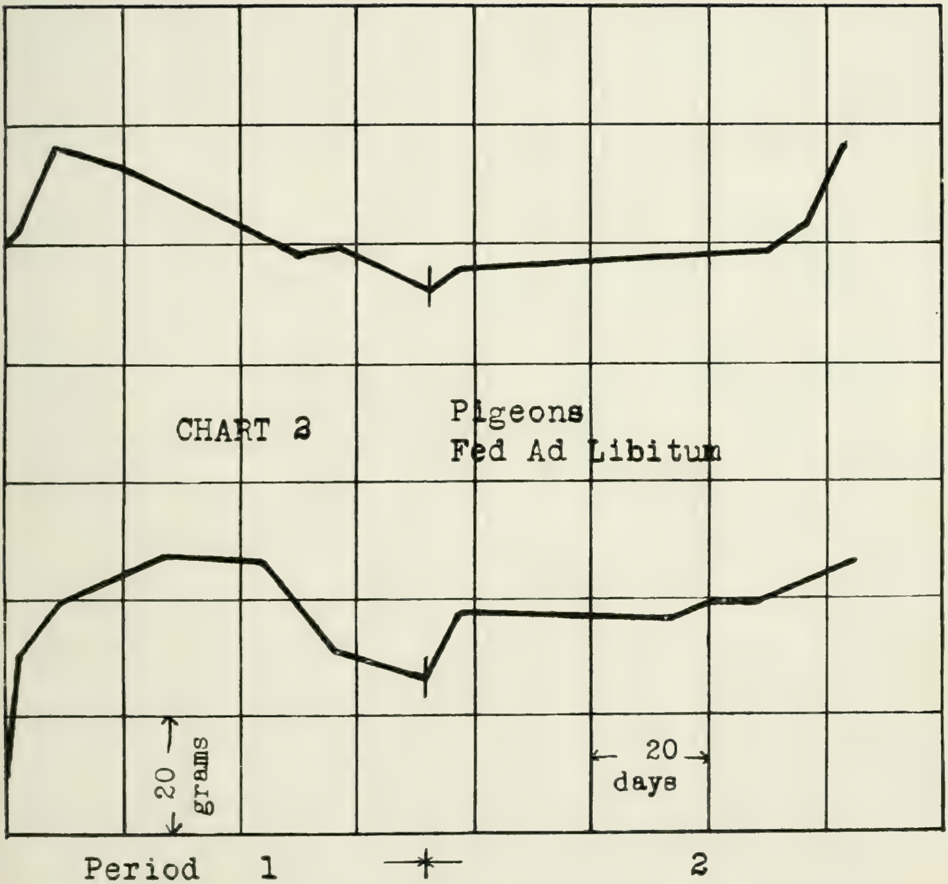

CHART 2. Shows the average weight curves for pigeons that were fed *ad libitum*. Period 1 unmilled rice heated for 2 hrs. at 120° C. in the air oven and Period 2 on the rice that was heated in the autoclave at 120° C. There was a gradual loss in weight on the dry heated rice, and only slight symptoms of polyneuritis. Following this, the pigeons gained slightly on the 1 hr. autoclaved rice, just as they did in Chart 1.

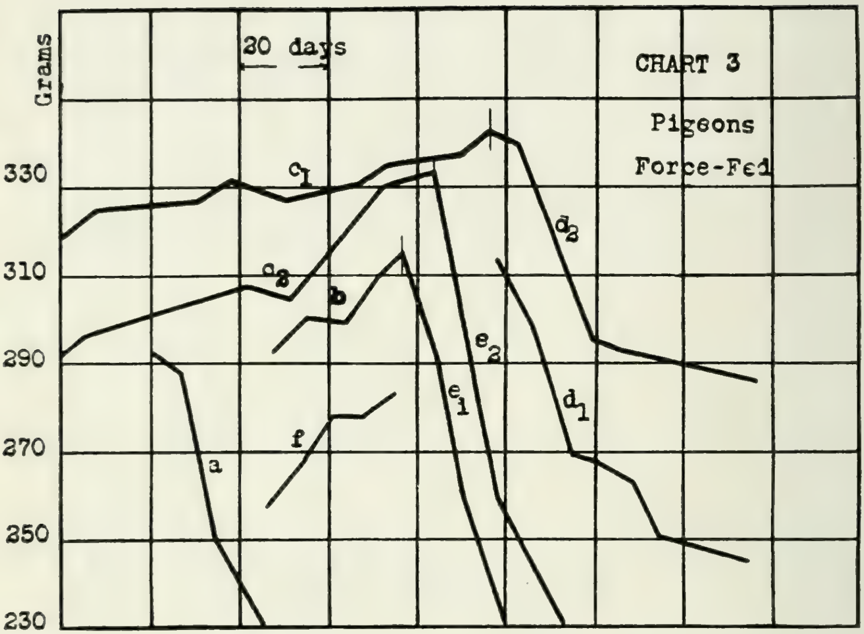

CHART 3. Pigeons were first given *ad libitum* unheated and 1 hr. autoclaved rice (Curves b, c_1, and c_2), respectively. After a time they were force-fed, in some cases on the 2 hrs. autoclaved rice and in others on the 6 hrs. heated rice (Curves d_1, d_2, e_1, and e_2, respectively). In Curve a, they were force-fed the milled rice as a control.

When they consumed the unheated rice they gained, and lost rapidly when force-fed on the 6 hrs. heated rice. Likewise, they gained on the 1 hr. autoclaved rice and lost on the 2 and 6 hrs. heated rice. The pigeons regurgitated some of their food when force-fed, but retained more of it than when they were fed *ad libitum* in Chart 1. That this loss in weight was not due to the mechanical handling of the pigeons in force-feeding them is evidenced from Curve f, where they were force-fed on unheated unmilled rice and gained.

The curves show that even though the pigeons consumed more of the 2 and 6 hrs. autoclaved unmilled rice than when fed *ad libitum*, the extra amount had no value.

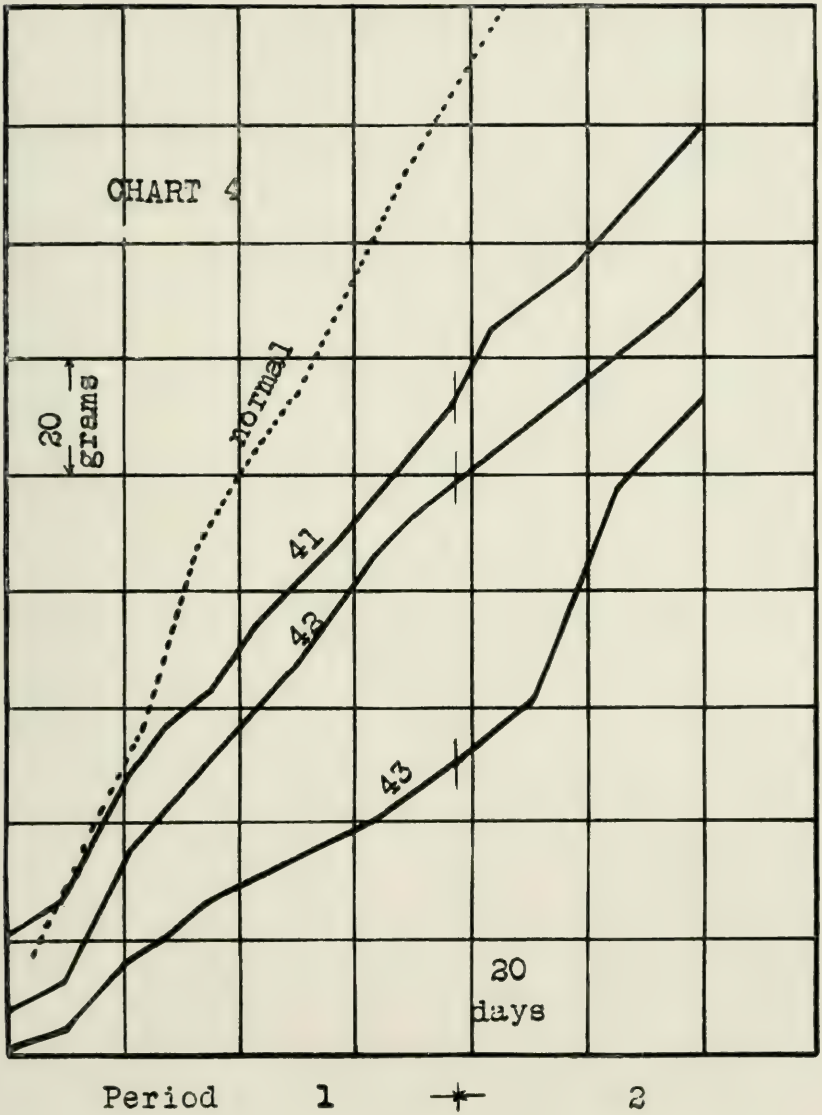

CHART 4. The rats were fed the basal diet of unheated unmilled rice, supplemented with lactalbumin, salt mixture, butter fat, and lard. The protein plane was 10 per cent. In Period 1 the rice formed 64 per cent of the ration and in Period 2 89.7 per cent.

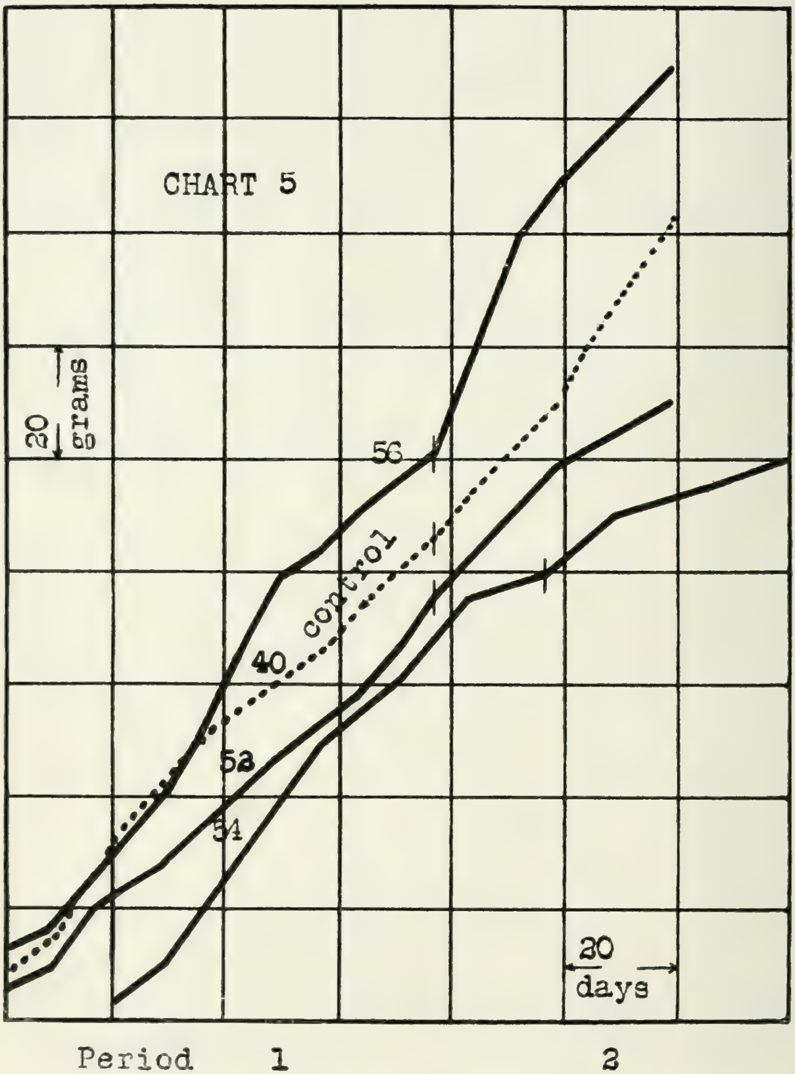

CHART 5. The rats were fed the basal diet of unmilled rice heated 2 hrs. in the air oven at 120° C. Otherwise the ration was the same as for Chart 4. The control, Curve 40, is the average for the curves for the unheated rice (Chart 4).

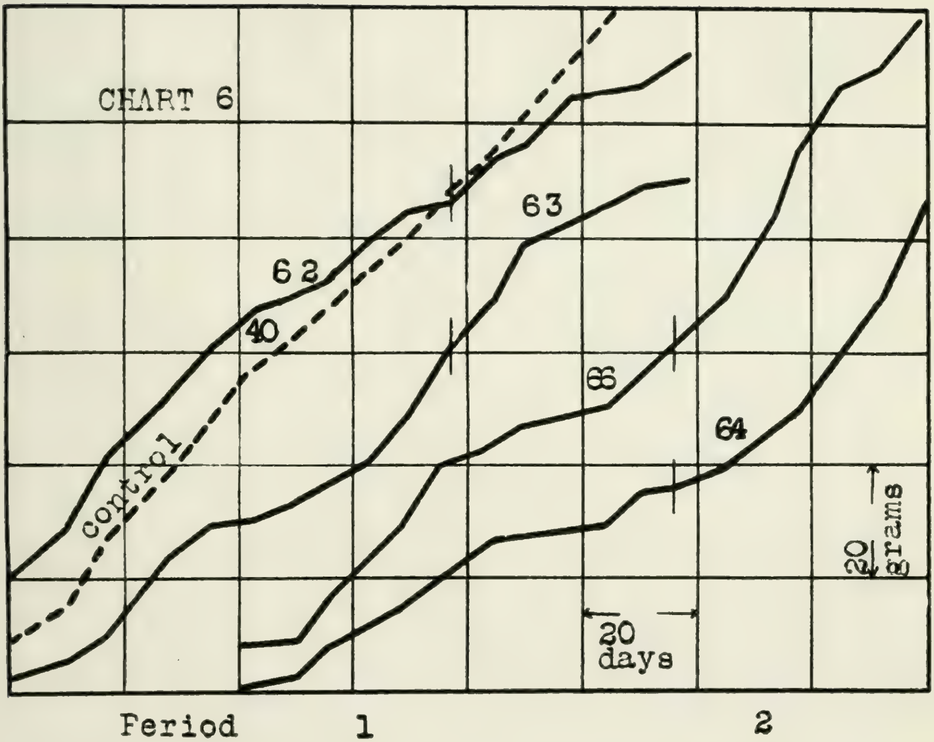

CHART 6. Same as Chart 5, except the basal rice diet was heated 2 hrs. in the autoclave at 120° and 15 pounds pressure.

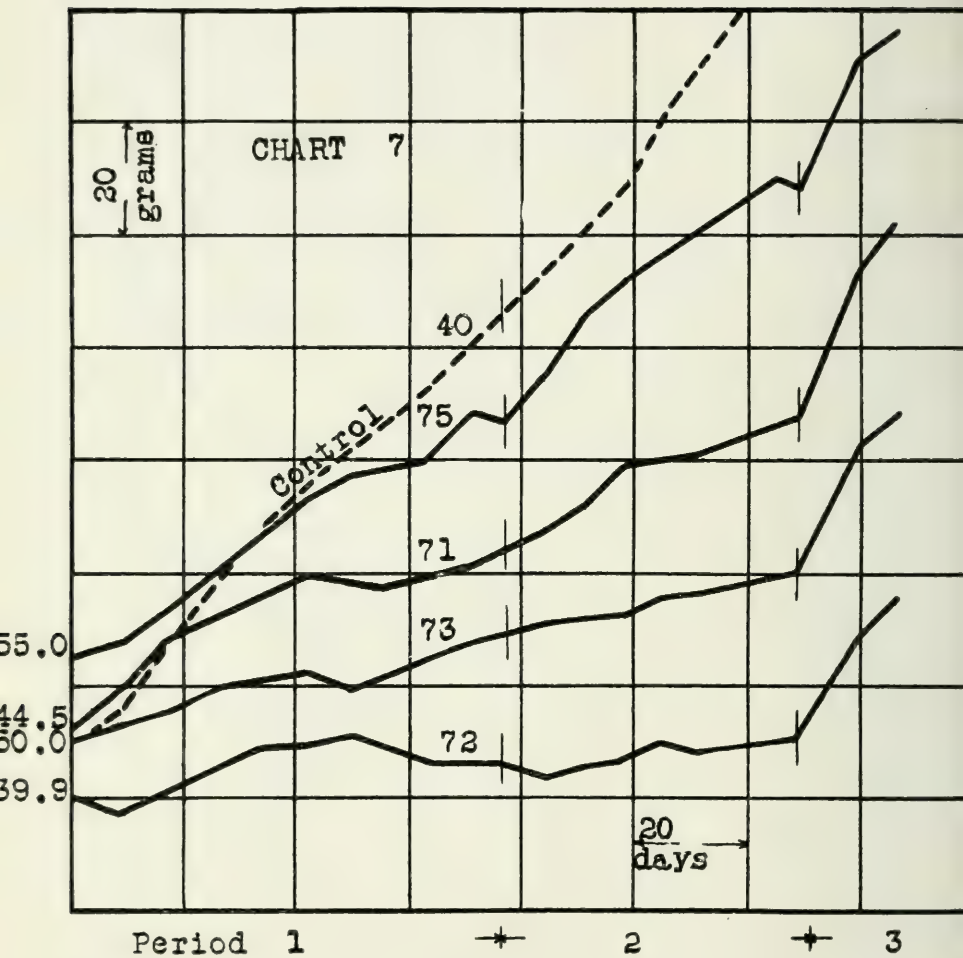

CHART 7. Same as Chart 6, except the rice was heated for 6 hrs. in the autoclave at 120° and 15 pounds pressure. In Period 3, the rats were fed the same ration as in Chart 4.

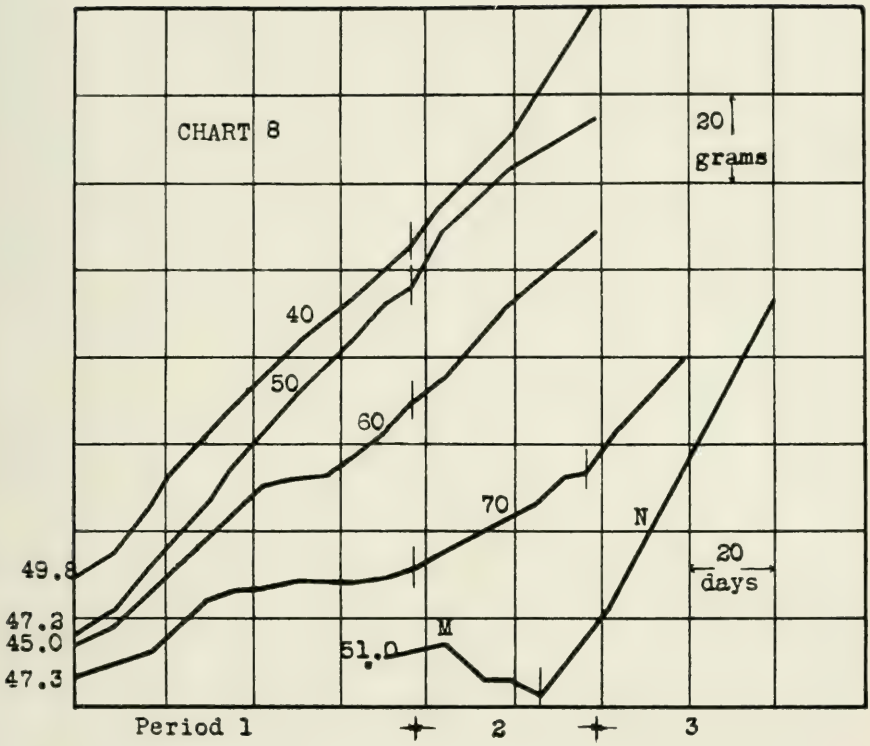

CHART 8. The average curves show that the growth-promoting vitamin was not altered by heating at 120° for 2 hrs. in the oven. Thus, Curve 50 ran parallel with that for the unheated rice (Curve 40). In the case of the 2 hrs. autoclaved rice, heated at 120° and 15 pounds pressure, Curve 60 ran practically parallel with the other two, except for a short period. The curve for the 6 hrs. heated rice (Chart 70) shows some gain. In all four groups there was a definite gain.

If the growth-promoting water-soluble vitamin had been destroyed by heat, the resultant effect in these four curves would have been a decline in weight the same as for the first part of the control, Curve *MN*, where rats were first (*M*) fed on a ration composed of butter fat, lard, starch, lactalbumin, and salt mixture without any of the so-called water soluble B, and later (*N*) given this vitamin.

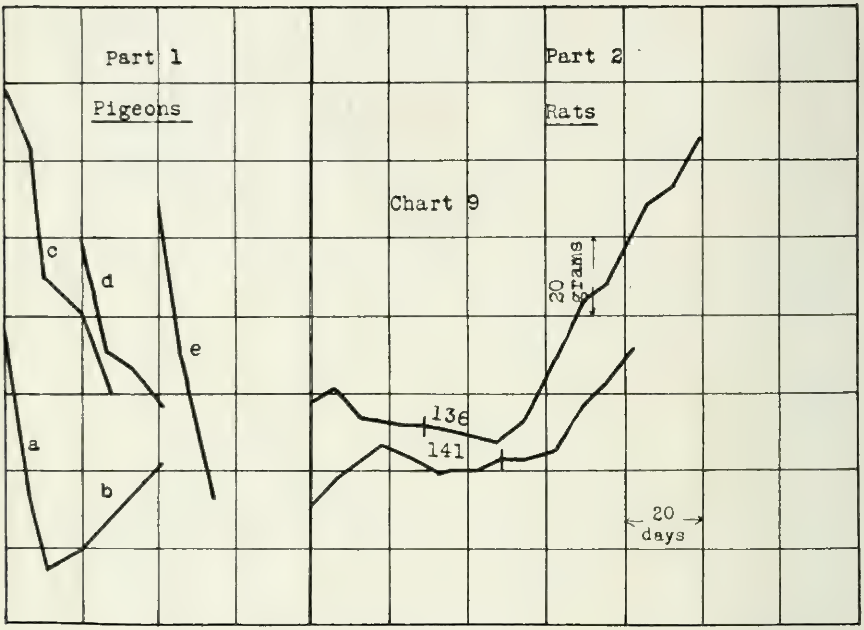

CHART 9. In Part 1, the pigeons were all force-fed milled rice. In the case of Curve a, they were not treated until there were definite signs of typical polyneuritis. Then the pigeons were treated with unheated vitamin extract. In Curves c, d, and e, the pigeons were treated from the beginning every other day with equivalent amounts of the extracts that had been heated 2 hrs. at 120° in the air oven, 2 hrs. in the autoclave, and 6 hrs. at 120° in the autoclave, respectively. The evidence is quite conclusive that heating under these conditions destroyed the vitamin. In fact, the vitamin in the extract was undoubtedly broken down more quickly than in the rice.

In Part 2, the same vitamin extract after heating 6 hrs. in the autoclave at 120° was incorporated in a ration that was deficient in the water-soluble growth-promoting factor. The fore period shows that the rats were not growing, while the test period indicates that they began to grow after adding the extract, indicating that this vitamin was not totally destroyed by the heating.

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 258, 1920.

(Reprinted from *The Journal of Biological Chemistry*, Vol. XLIII, No. 1, 1920.)

WATER-SOLUBLE VITAMINS.

II. The Relation of the Antineuritic and Water-Soluble B Vitamins to the Yeast Growth-Promoting Stimulus.*

By A. D. Emmett and Mabel Stockholm.

(From the Medical Research Laboratories of Parke, Davis & Company, Detroit, Mich.)

With the idea of making a further study (1) as to whether the antineuritic and the water-soluble B vitamins were different, we endeavored to apply one of the two yeast quantitative methods that have been proposed. We selected the Williams (2) micro method which the author claims measures qualitatively and quantitatively the presence of the antineuritic vitamin. Bachmann's fermentation method (3) has been used by Eddy (4).

The plan of approaching this phase of the problem was similar in some respects to that which we used formerly (1); that is, a study of the effect of heat on the water-soluble vitamins. Comparative trials were made to determine whether or not the extracts that caused increased growth of the yeast would cure polyneuritis in pigeons, or excite growth in young rats that were suffering from a lack of the water-soluble B vitamin.

Briefly, this micro method[1] was as follows: (*a*) Preparation of synthetic media—saccharose, 20 gm.; $(NH_4)_2SO_4$, 3 gm.; KH_2PO_4, 2 gm.; asparagine, 3 gm.; $CaCl_2$, 0.25 gm.; and $MgSO_4$, 0.25 gm., all made up to 1 liter with distilled water. The reagents were purified. The media was sterilized at 10 pounds pressure for 10 minutes and kept in the refrigerator. (*b*) Procedure: A suspension was made of yeast cells in about 30 cc. of sterile distilled water. Duplicate test solutions were prepared quantitatively by taking 25 cc. of the synthetic media and 1 to 5 cc. (depending upon the concentrations) of a definite solution of the unknown extract. This was diluted to 30 cc. (using sterile water if needed). The mixture was sterilized and 1 cc. of the yeast suspension added. After mixing, thirty-six drops were made with a fine pen point on a cover slip that had been coated

*Proceedings of the American Chemical Society, St. Louis, April, 1920

[1]The authors desire to express their appreciation to Professor F. C. Koch, of the University of Chicago, for the privilege of permitting us to become familiar with the technique of the details of this method.

with a very thin film of purified vaseline. This slip was inverted and sealed tightly on a hanging drop slide. Each drop was examined for single yeast cells, the observations were recorded, and the time was noted. The slide was then placed in the incubator at 30°C. and the readings were made again in 18 hours. In case the number of cells in each drop at the end of this period exceeded 75, the determination was repeated, using less of the unknown. Similar tests were run on the synthetic media as a control. After correcting for this blank determination, the rate of growth in terms of yeast cells was calculated per gram of the original unknown material.

Table I gives an idea as to the accuracy of the Williams method. It will be seen, in varying the concentration of the solution, or in taking different quantities of the same solutions, that not only the duplicates in any one series but the corresponding final average values in the different series agreed remarkably close for such a biological method, showing that this method can be used with definiteness for measuring the rate of growth of yeast.

DISCUSSION.

The Yeast Growth-Promoting Factor Is Not the Antineuritic Vitamin.—In making these tests, natural or unmilled rice was

TABLE I.

Preliminary Tests with the Williams Micro Yeast Method.

Concentration of solution of vitamin extract No. 2650.	Amount added to media.	Average reading of yeast cells.*	Yeast cells per gm. of original substance.	Average of duplicate cells per gm. of substance.
per cent	*cc.*			
A. 0.02 made from a 5 per cent	1.0	7.8	39,000	
solution by dilution.	1.0	8.1	40,500	39,750
B. 0.02 made from a 5 per cent	3.0	23.8	39,500	
solution by dilution.	3.0	23.6	39,000	39,250
C. 0.02 made directly.	3.0	23.6	39,000	
	3.0	24.0	40,000	39,500
D. 0.2 made from a 5 per cent	3.0	82.2	38,600	
solution by dilution	3.0	82.4	38,700	38,650
Average				39,287

* Corrected for control reading on reagents.

heated as follows: 1 hour in the autoclave at 120° and 15 pounds pressure; 2 hours in the autoclave at 120°; and 6 hours in the autoclave at 120° and 15 pounds pressure. These rices, together with some of the unheated rice, were finely ground, then extracted with hot 95 per cent alcohol. These extracts were concentrated *in vacuo*, taken up with hot water and salt, then filtered, and made up to a definite volume. The tests were then made upon these solutions, blank determinations being carried out with the same dilution of salt and synthetic media. Corrections were applied in making the final calculations. No data are included with respect to comparing heated extracts that originally contained the antineuritic vitamin. We found that this extracted vitamin was apparently more unstable to heat than when in the natural food.

The data are presented in Table II. They indicate very clearly that the factor which stimulated growth in the yeast was not altered in the least by the process of heating. This observation was contrary to what we had expected, since Williams (2) designated the yeast growth stimulus as the antiberiberi vitamin. From previous findings (1) we have already concluded that the extensive heating of the rice (2 and 6 hours in the autoclave at 120° and 15 pounds pressure) altered in a very marked way the

TABLE II.

Effect of Heat on Yeast Growth-Promoting Vitamin

Extracts of natural rice	Average reading of each determination.*			Average yeast cells per cc.	Yeast cells per gm. of original rice.
	1 cc.	3 cc.	4 cc.		
Unheated rice.	12.7	38.0			
	12.3	38.7		12.6	63
1 hr. in autoclave at 120°C.	12.9				
	13.1			13.0	65
2 hrs. " " " 120°C.	12.8	37.7			
	12.9	37.1		12.6	63
6 " " " " 120°C.	13.1		49.6		
	13.1		49.0	12.7	64

* Corrected for control reading on reagents.

potency of antineuritic vitamin. Therefore, we naturally expected the activity of the extracts, in terms of yeast cells, to decrease with the higher degree of heating.

In order to prove this point more definitely, the antineuritic property of the extracts of these rices was tested by treating polyneuritic pigeons with equivalent quantities of each extract in terms of the number of cell units per gram of body weight These data are presented in Table III.[2]

TABLE III.

Potency of Extracts from Natural Rice on Polyneuritic Pigeons.

Extract of natural unmilled rice	Pigeon No.	Equivalent yeast cells given in extract.	Yeast cells per gm. of body weight.	Response to treatment.
Unheated	510	10,269	41	Cure.
	803	7,812	31	"
Average.			36	"
2 hrs. in autoclave	805	9,750	38	No improvement, died.
at 120°C.	793	9,750	38	" " "
Average.			38	" " "
6 hrs. in autoclave	735	10,400	42	No improvement, died
at 120°C.	781	10,400	33	" " "
Average.			38	" " "

There is, of course, a possibility that in the heating of the rice some toxic substances were formed which were removed in the extraction along with the vitamin and in turn prevented the antineuritic vitamin from acting on pigeons. If so, one would expect the yeast growth-promoting vitamin to be affected in the same manner, but it was not. On the other hand, with the yeast cell stimulus, we have found that some extracts contain toxic factors although the antineuritic and the water-soluble B vitamins were shown to be present by biological tests. As a result, the readings were very misleading as shown in Table IV.

[2]In making these tests the pigeons selected showed the usual definite signs of typical advanced polyneuritis. The "cure" cases remained positive for 7 to 10 days, which is sufficient for a definite test of the potency of an extract.

The Yeast Growth-Promoting Factor Apparently Does Not Stimulate Growth in Young Rats.—In order to obtain an idea as to the amount of the yeast stimulus that normal rats require, the

TABLE IV.

Toxicity in Relation to the Yeast Growth-Promoting Stimulus.

No. of extract.	No of yeast cells in diluted solution.	
	1 cc.	3 cc.
30–40	$\underbrace{12.7\text{–}12.8}_{12.75}$	$\underbrace{7.8\text{–}8.7}_{8.25}$
30–50	$\underbrace{10.9\text{–}11.0}_{10.95}$	$\underbrace{7.3\text{–}8.9}_{8.1}$
1,339 (2 hrs. in autoclave at 120°C.)	$\underbrace{9.5\text{–}13.5}_{11.5}$	11.9
1,339 (6 hrs. in autoclave at 120°.)	$\underbrace{13.2\text{–}13.9}_{13.55}$	$\underbrace{11.7\text{–}11.9}_{11.8}$

value was calculated from previous prophylactic feeding experiments where rats were fed as their basal diet natural rice (1). The data are given in Table V.

In making the tests to determine whether or not the rats would

TABLE V.

Calculated Yeast Cell Units Consumed by Normal Rats.

Substance.	Group of rats.	Yeast cell units per day per gm.	Remarks.
Natural rice, unheated.	40	3.0	Gained in weight.
" " 2 hrs. dry heat at 120°.	50	3.3	" " "
Natural rice, 2 hrs. autoclaved at 120°.	60	3.3	" " "
Natural rice, 6 hrs. autoclaved at 120°.	70	2.7	Moderate gain in weight.

be stimulated to grow on a definite number of yeast cell units, a water-soluble vitamin preparation was activated with fullers' earth by the Seidell method (5). The filtrate was found to contain 2,537 yeast cell units or 6.4 per cent of the total yeast factor. Failure to cure pigeons showed that its antineuritic vitamin content was extremely low.

Rats that had been brought down to a low nutritive plane, due to a lack of the so called water-soluble B, were treated with 1 to 1.5 cc. of this filtrate. As the rats were weighed daily, it was found (Table VI) that Rats 834 and 835 both lost in weight during the 15 days' treatment. Further, in the case of Rat 735, its loss in weight was so marked that we were forced to give it a treatment of the original vitamin extract, whereupon it immediately began to gain.

TABLE VI.

Relation of Yeast Cell Units to Growth of Rats.

Substance.	Rat No.	Yeast cell units per day per gm.	Remarks.
Extract 7,000.5b-Filtrate fullers' earth.	834	7.0	Weighed 47 gm. Lost 4.2 gm. in 15 days.
Extract 6 hrs., autoclaved 120° natural rice.	834	7.5	Gained 3.0 gm. in 5 days.
Extract 7,000.5b-Filtrate fullers' earth.	835	7.8	Weighed 35.5 gm. Lost 4.0 gm. in 15 days.
Extract 6 hrs., autoclaved 120° natural rice.	835	6.9	Gained 10 gm. in 5 days.
Extract 7,000.5b-Filtrate fullers' earth.	735	15.3	Weighed 49.5 gm. Lost 6.4 gm. in 3 days.

Following the first treatment of Rats 834 and 835 with an extract from the 6 hours autoclaved 120° natural rice, they stopped losing and began to gain on doses that were equivalent in yeast cell units to what they were given of Extract 7,000.5b.

In Table VII, further data are presented with respect to the amount of extract, in terms of yeast cell units, that were required

to make a rat grow. These two rats, Nos. 673 and 675, were so far down from a lack of the water-soluble B that they had reached that condition which is described by some as polyneuritis.

It is thus seen that the rats in the curative or corrective tests (Tables VI and VII) were given 2 to 2.5 times as much of the yeast cell units in Extract 7,000.5b as those on the prophylactic tests (Table V), yet they did not respond and grow. This suggests that either the yeast cell stimulus was not the same as the water-soluble B, or that the rats in this condition required more of it. When the dose was increased to almost five times, Rat 735 refused to grow. This led us to infer that there was some factor involved in the growth of the rat other than the yeast cell units, or at least to conclude that the yeast cell stimulus was not in itself able to retard the loss in weight and excite growth, measured by increase in weight of rats. As a further proof of this, the data in Table VII tend to show that, with the amount and kind of extract used, the water-soluble B vitamin had a specific action on the growth of the rat that the yeast stimulus lacked.

This statement is not intended to convey the idea that the stimulus or vitamin which certain varieties of yeast seem to

TABLE VII.

Relation of Yeast Cell Units to Growth of Rats.

Substance.	Rat No.	Yeast cell units per gm.	Remarks.
Extract 2 hrs. autoclaved 120° natural rice.	673	14*	Weighed 7.2 gm. Lost 9.2 gm. in 5 days.
Extract unheated natural rice.	673	14	Gained 25 gm. in 19 days, following one treatment only.
Extract 2 hrs. autoclaved 120° natural rice.	675	14*	Weighed 99.7 gm. Gained 11 gm. in 5 days.
Extract unheated natural rice.	675	14	Gained 20 gm. in 19 days, following one treatment only.

* Given every 2nd day.

utilize is not needed by the rat or the pigeon for some other definite purpose or purposes in their physiological economy. Our data do not lend themselves to an interpretation of this point.

CONCLUSIONS.

1. The rate of growth of the yeast which we employ is strikingly accelerated by the addition to the synthetic media of very small amounts of preparations, which we have shown by biological tests to contain the antineuritic and the water-soluble B vitamins, provided certain toxic substances are absent.

2. The yeast growth-promoting factor does not appear to be the same as the antineuritic or antiberiberi vitamin (pigeons).

3. This yeast stimulus is also possibly different from the water-soluble B growth-promoting vitamin (rats).

4. Whether pigeons or rats require this yeast growth-promoting factor for normal development has not, as yet, been definitely proved.

5. Since the amount of yeast growth stimulus, expressed in terms of yeast cell units per gram of substance, does not appear necessarily to vary directly (in terms of potency) with the antineuritic and water-soluble B vitamins, this yeast method should not be employed quantitatively with too much definiteness until further study is made.

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Studies from the Medical Research Laboratories,
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Reprint No. 259, 1920.

(Reprinted from *Science*, N. S., Vol. LII, No. 1337, pages 157-158, August 13, 1920.)

THE FAT-SOLUBLE A VITAMIN AND XEROPHTHALMIA.[1]

By A. D. Emmett.

(From the Medical Research Laboratories, Parke, Davis & Co., Detroit, Mich.)

It is generally admitted by those who have conducted feeding experiments with rats that although the essential dietary factors for growth, including the so-called water-soluble B, are present, the animals will not grow to maturity without the fat-soluble A. The work of Osborne and Mendel, McCollum and associates, Drummond, Steenbock and associates, and others give abundant evidence of this fact. All investigators are not in accord, however, that a positive lack of the fat-soluble A is the direct cause of the eye condition in the rat which McCollum[2] designated as xerophthalmia, some considering this disease to be primarily infectious.

Bulley[3] has recently taken the most definite stand that this eye condition is not due to a dietary deficiency but primarily to infection, resulting from poor hygienic surroundings and uncleanliness. She based her conclusions on a study of some 500 rats that were fed on definite synthetic rations.

In our laboratory we have had occasion to feed white, and black and white rats on various synthetic rations, and in going over our records we have compiled data bearing upon the prevalence of xerophthalmia in relation to the known presence or absence of the fat-soluble A. These results are given in the table below.

Group	Vitamins Absent from Ration	Number of Rats Reported	Positive Cases, Xerophthalmia	Per Cent. Positive Cases
A	Fat-soluble	122	120	98.3
B	Water " *B*	103	None	None
C	None (controls)	216	...	"

[1]Read before the American Chemical Society, St. Louis, April, 1920.
[2]McCollum, E. V., and Simmonds, N., *Jour. Biol. Chem.*, 1917, XXXII, 29.
[3]Bulley, E. C., *Biochem. Jour.*, 1919, XIII, 103.

It is seen that out of 122 rats, Group A, 120 of them, or 98.3 per cent, showed sooner or later positive signs of xerophthalmia, and that when the fat-soluble A vitamin was present, with or without the water-soluble B (Groups *B* and *C*), none of these 319 rats showed evidence of this eye ailment. All the rats were fed individually in practically every case. They were kept in metal cages, without any bedding, which were provided with a special removable wire screen floor. The cages and the food and water cups were always disinfected once or twice a week. The sanitary conditions were, therefore, good. The same assistants handled and fed all the rats, so that the attention given them was the same for all, and the possibilities of infection from this source were uniform.

It would seem to us that if xerophthalmia was primarily infectious and due to the poor hygienic conditions, some of the rats in Groups *B* and *C* would certainly have developed it. Further, repeated attempts were made to transmit the disease by using sterile threads of gauze, passing them cautiously over the edge of the lids of the sore eyes, and then carefully inoculating the eyes of the other rats. These tests were negative, as were the controls. This was fairly good evidence that the disease could not be transmitted by this means.

Treatment of advanced cases of sore eyes with a saturated boric acid and also with a silver protein solution failed to relieve the condition. However, when as little as 1 to 2 per cent. of an extract containing the so-called fat-soluble A vitamin was added to the ration, the eyes were speedily cured and the rats increased in weight, indicating that this extract was a specific cure for xerophthalmia.

We therefore agree with McCollum, that xerophthalmia is primarily a dietary deficiency disease, due to a lack of the fat-soluble vitamin. The certainty of the prevalence of the disease depends on the high purity of the essentials that enter into the ration, and on the length of time of feeding, younger animals showing the symptoms much sooner than older ones.

Acknowledgment should be made of the assistance rendered by Miss Marguerite Sturtevant in carrying on this project.

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 260, 1920.

(Reprinted from the *American Journal of Pharmacy*, Vol. 92, No. 8, August, 1920.)

THE BIOLOGIC METHODS FOR DIGITALIS ASSAY.*

By Herbert C. Hamilton.

(From the Medical Research Laboratories, Parke, Davis & Co., Detroit, Mich.)

There are many steps in the process of transforming the lovely old fashioned flowering plant, foxglove, into the powerful medicinal product known as the digitalis heart tonic. In fact, some of the steps might be called stopping places and regarded as of more than passing interest; especially such points as the selection of some one out of the many varieties of digitalis to be the official one, the choice of the portion of the plant to be used, the age of the plant, and the menstruum for extraction. The seriousness of these problems may be judged by the last mentioned, since the U. S. P. Revision Committees have, on each occasion, selected a different menstruum for extracting the crude drug.

But of all the problems demanding attention, probably no one has received more than that of proper means of testing and standardizing the crude drug and its extracts. From the time of Fagge and Stevenson's work[1] recorded in 1866 up to the present time, the controversy has raged unceasingly.

These investigators first used the method later modified and known as the Focke method,[2] by which the frog was injected in the thigh, the heart exposed and the time noted when the heart stopped in systole.

Koppe, in 1875,[3] studied the action on warm-blooded animals, noting the effect on the heart, but especially the amount necessary to induce vomiting. This was later suggested by Hatcher[4] as a means of standardization.

Bennefeld,[5] in 1881, in his careful examination of tinctures of digitalis from different parts of Germany, selected the method later known as the Hatcher Cat Method,[6] the diluted preparation being slowly injected into the vein of a rabbit until death occurred.

*Paper read at the St. Louis meeting of the A. C. S. and published by courtesy of the American Chemical Society.

Bardet,[7] in 1889, used the frog in the way later suggested by Houghton, that is, comparing activities on the basis of the minimum lethal dose.

Houghton,[8] in 1898, first published the method and described the exact technic by which to standardize the digitalis preparations. The method, at time of publication, had actually been in practice for three years and is the first recorded attempt not merely to test but really to establish uniformity in preparations of this drug.

Prevost,[9] in 1893, used frogs and chose as end-point the systolic stoppage of the heart.

Fraenkel,[10] in 1902, was the first to apply this method, now official in the U. S. P.,[11] as an exact method for quantitative assay, selecting one hour as the time for systolic stoppage.

Focke,[2] in 1902, and frequently at later dates, wrote on the use of frogs for standardizing digitalis. He selected the systolic stoppage in 7 to 10 minutes as the end-point and suggested a formula by which to deduce the value from the data, including the weight of the frog, the dose and the time of systolic stoppage. He has written voluminously on the subject.

From this time on no absolutely new method has been devised, the research being confined to modifications and adaptations of the methods proposed by earlier workers.

Such is the Hatcher Cat Method,[6] the Reed and Vanderkleed Guinea Pig Method,[12] the M. S. D. Frog Heart Method,[11] and the Pittenger Gold Fish Method,[13] although the latter is the only published one using this test object.

Laying aside for the moment the question as to the validity of any of these methods, we should attempt to consider, in a broad way, the more or less derisive question, "Is the sample of drug that has been found to possess the greatest power to kill a cat, the one that will prove most efficient in curing a man?" Rusby[14] is skeptical of such methods because as a botanist he is not fully informed as to their applicability.

Lloyd,[15] perhaps for the same reason, says in reference to a toxicity experiment recorded by Withering: "In those days of heroic medication it was naturally concluded that a drug that could thus kill a turkey must be good medicine to cure a human being, a process of reasoning not yet altogether obsolete."

Beal,[16] with no circumlocution but directly to the point, says: "If we would know the physiologic action of a drug upon the human we must observe its action on the human; this cannot be deduced with any degree of certainty by its action on one of the lower animals."

All these comments are based on a wrong conception of pharmacology. While there are occasional instances where the action of a drug on one animal differs fundamentally from that on another or on a human, such anomalies are usually traceable to fundamental differences in the two species. To illustrate, the cat and dog vomit easily, the guinea pig not at all. The frog will not respond to the action of a febrifuge since its temperature is always that of the surrounding medium. But all, including man, have the heart and the circulatory system which either directly or through the nerves are acted upon by external influences such as drugs. Certain features of these effects may differ, but fundamentally the fact remains unassailable.

We therefore take issue with Beal to this extent, that while there are unquestionably cases in which the action of a drug on man differs materially from that on certain animals, these are exceptional cases.

Practically every physiological reaction in man has its counterpart in some animal.

It is on this basis that the science of pharmacology is founded. Through the investigation of the action of drugs on animals many of their valuable properties have been discovered; by this means most of these actions have been explained. Digitalis acts as a diuretic. Is it a direct action on the kidneys or is it only an indirect effect of its action on the circulatory system? By animal experiment and not by observation of humans this has been made clear. Digitalis has a strongly tonic action on the heart and vessels. Is its action directly on the muscles or is this an effect of its action on the nerves? Again we are rewarded by the answer as a result of animal experiments.

But the critic is perhaps still dissatisfied. Granted that all this is true he yet is skeptical on one or two points. How is it possible to standardize drugs even if the action on some animal is apparently similar to its action on man? This is a logical question and deserves an answer.

Digitalis administered to animals in doses comparable to the therapeutic dose for man does not show a measurable effect except when such doses are given intravenously to an anesthetized animal, the effect being measured by the aid of a myocardiograph or similar instrument—a measurable effect sufficient for qualitative purposes. For a quantitative test comparison must be made with a standard just as the chemist must have absolutely pure reagents or must be able to discount the impurities by running a blank or some similar procedure.

Digitalis will slow the beat of the heart, increase the amplitude of the beat, raise the blood pressure, but the amounts necessary to bring about a definite degree of change in these measurable reactions differ with different dogs, so the effect of the sample on one dog cannot be compared with the effect of the standard on another dog. By use of a number of dogs an average could be selected, but time and the expense of labor and animals will not permit this procedure. Again, to compare sample and standard on the same animal by means of consecutive injections is not practicable because the elimination of digitalis is so slow that the animal never fully returns to its original condition to permit comparing two injections even of the same sample.

For these reasons, attempts to use this as the end-point have been abandoned, and the death of the animal, or in the case of frogs stoppage of the heart in systole, selected.

Not only, therefore, can the action of drugs on the human be deduced from that on the lower animals, but also the degree of the action can be measured and the exact manner of action explained.

Lloyd's statement in reference to killing a turkey and curing a man may have been true in those days when the science was undeveloped. At present, however, the fact of killing is of little importance; the vital points in the experiment are the amount that killed, the action of smaller doses, and the possibility of applying the substance clinically to relieve a pathological condition.

There is no known medicinal substance which, taken in excessive quantities, does not induce toxic symptoms and, in most cases, death. It is not valuable because of this but in spite of it.

The question raised by Rusby is entirely logical and *apropos.* If a substance is standardized solely on the basis of its M. L. D. with no regard to the characteristic effect which that substance

may be expected to produce, common sense suggests that such a test is inadequate and should be used only if no verifying effect is available. We will attempt to apply this test of adaptability to the various methods for the assay of the digitalis series, disregarding the question of accuracy, and keep in mind only whether the test shows digitalis glucosides and no other poison to be present.

The Gold Fish Method, while little known or practiced, is a proposed means of assaying digitalis by its toxic action on goldfish, the drug being mixed in various dilutions with the water in which they swim. The end-point is the dilution which kills after a certain period of contact and with due regard to certain factors, such as the temperature of the medium. It has the advantage of being one of the simplest and probably cheapest of all. But for the fact that there is nothing typical of digitalis in the action of the drug on the test animal there is no reason why it should not have a favorable consideration. The goldfish, however, is exceedingly sensitive to substances not always recognized as poisons and certainly not in the same class with digitalis. While the chances of a foreign and more toxic substance being found with digitalis are remote, they are always possibly present as every manufacturer knows. Without a confirmatory test, the goldfish M. L. D. method is to be classed as questionable.

The guinea-pig method is no less open to question on the same score, although the results obtainable purely on toxicity are quite uniform and accurate. In this instance, also, there is no observable reaction characteristic of digitalis either before or after death; and death often results from respiratory paralysis rather than a direct action of digitalis on the heart.

The method is simple and as described by the authors inexpensive, since the intention is to use guinea-pigs already used for another purpose for which they no longer are eligible, and therefore with a value theoretically nil.

Injection is made subcutaneously and results recorded after a definite period. Results show that pigs, while fairly uniform, have individual variations as well as seasonal, but these can be eliminated as factors in the assay, by the occasional test of the standard and by the use of a number of pigs to obtain the average M. L. D.

The Hatcher Cat Method seems to have even less to recommend

it since there is nothing characteristic of digitalis in the death of the animal, and further, its inaccuracies have been the subject of comment by several authors. Hatcher[6] noted that in some cases cats required a dose 50 per cent larger than the average M. L. D. Robinson and Wilson[17] found in a series of ten cats that the M. L. D. ranged from 70 to 210 per cent of the average M. L. D. Eckler[18] found a variation of over 100 per cent in the M. L. D. of cats.

The details first published by Hatcher for the assay of digitalis required that the immediate cause of death should be ouabain; only a part of the M. L. D. was to be caused by the digitalis. The cat is partly anesthetized in order to open a vein into which is inserted a glass cannula. Through this the injection is made slowly over a stated period. The animal must die in less than 90 minutes. The method has been modified by most investigators, especially by those who found any valuable feature in it.

The only radical change, however, is that made use of by Newcomb of the Pharmacy Department of the University of Minnesota. It is commonly assumed that Newcomb uses the Hatcher Method without material change from that first published, and this opinion will perhaps prevail until some published account of his work appears. Unofficially, however, certain statements have been made such as these: "Hatcher's method, without modification, is of little value." "The cat is the least important part of the method." "The important part of the method is the observation of the action of digitalis on the bundle of His."

One may assume, therefore, that Newcomb discredits Hatcher's Method, in which the death of the cat is the deciding factor, and pins his faith on observing the heart block which follows fatigue of the auriculo-ventricular bundle. This is, to a limited extent, characteristic of digitalis poisoning and so may be regarded as a valuable feature to distinguish the *character* of the poisoning, but it adds nothing to the accuracy of the only quantitative features which the test possesses, namely, the M. L. D. (minimum lethal dose).

As a qualitative test this feature is admirable; but no pharmacologist has succeeded in making the test an accurate quantitative one unless as yet unpublished.

The M. S. D. Frog-heart Method—the Pharmacopœial method

—is, in brief, to inject the diluted preparation into the abdominal lymph sac of the frog, injecting them in series of three and at the end of one hour exposing the heart to observe its condition. The end-point is that at the end of the one-hour period the heart must be stopped in systole while the next smaller dose—a difference not to exceed 10 percent—leaves the heart beating at that time. This method has two advantages over those employing mammals, namely, that a sufficient number of test animals can be used to detect and exclude those more or less resistant than the average and to test the standard on a number of similar animals under exactly the same conditions of weight, age, temperature and season, all of which may be variants. It has the further advantage that the end-point can be recognized as being due to one of the digitalis series, and to no other poison, by the position of the heart in systole.

It has the advantage over Newcomb's modification of Hatcher's method in the greater accuracy in the end-point by having a large number of test animals from which to exclude those of exceptional resistance—either high or low.

The M. L. D.[19] Frog-heart Method has for its end-point the minimum lethal dose—the smallest dose causing death with heart in systole.

The frogs are injected in the abdominal lymph sac with dilutions of the preparation, using such dilutions as to make a total dose less than 1 cc. The frogs consistently dying with this dose are examined to observe the position of the heart, which must be in systole, the same as by the one-hour method.

This has all the merits of the U. S. P. Method with the added advantage that in case of slow absorption the longer time limit permits, in most cases, total absorption. A further advantage lies in the fact that the frogs are not handled roughly, as in pithing and laying bare the heart, a procedure which may tend to influence the results adversely.

A third advantage is in the certainty of the end-point—death—as against the frequent occurrence of a heart at the end of the one-hour period not being positively stopped in systole.

Dr. Edmunds, of the Department of Pharmacology of the University of Michigan, and Dr. Worth Hale, Assistant Dean of the College of Medicine of Harvard University, were called to Washington to carry out such experiments as would determine, if pos-

sible, which of the methods is most practicable and reliable for the assay of digitalis. Their results and conclusions appear in *Hygienic Laboratory Bulletin No. 48.*[20]

Regarding Focke's Method, which is used mostly in Germany, they say: "However, even with these precautions we believe that this method allows of greater variations and inaccuracies than any other method we employed."

Attempts to apply a test by which the increase in blood pressure is used as the measurable reaction led to the following conclusion: "The blood pressure method upon cats and dogs commends itself on account of the close relation it sustains to the use of the drug in clinical practice. The objections consist in the difficulty of procuring these animals at times, and also the necessity of carrying out repeated tests to confirm the results which a study of our tables show will vary greatly."

As regards the M. L. D. and M. S. D. Frog Heart Methods, they found it impossible to choose with any degree of accuracy. "Between these two methods, as far as can be judged in the light of our present knowledge, it is largely a question of personal preference or convenience."

Cushny [21] ("Pharmacology and Therapeutics"), comparing the action of digitalis on frogs and mammals, says: "The effects of digitalis on mammalian heart therefore resemble in general those observed in the frog's. The contraction is not prolonged, however, as in the latter, and the inhibitory mechanism plays a more important rôle. . . . The heart in mammals is generally found in a condition of diastole in cases of fatal poisoning, and this has been supposed to indicate a fundamental difference in the action of digitalis on the amphibian and the mammalian heart. The dilatation is not, however, a direct result of the digitalis but is probably induced by the poisons formed in the heart by its own activity."

It is evident, therefore, that the frog shows a typical effect of digitalis just as distinctly as the mammal, that it can be used in numbers economically impossible in comparison with cats or dogs and thus overcome the factor of individual variations, and that the digitalis action differs in no material respect from that on mammals.

The very fact that the science of Pharmacology has made such

wondrous strides is evidence that the action of drugs on the human may be deduced from that on the lower animals.

The very fact that a system of dosage has been worked out for the powerful drugs shows that their action is measurable by the effects produced.

Few, however, seriously claim to be able to deduce human dosage of a new drug from its effects on experimental animals, except in the most superficial way, and with our present knowledge any such attempt will probably lead to failure.

It is, on the other hand, common laboratory experience to obtain practical working information in regard to a new drug by comparing the intensity of its effects with that of a similar known drug. If, for example, a special preparation of digitalis such as an active principle is produced, its good and bad properties can readily be deduced for human medication by comparing it with Tr. Digitalis on laboratory animals.

Or an entirely new local anesthetic can be compared with cocaine on animals and thus obtain actual data as to its effectiveness under most conditions, and its comparative toxicity, absorbability and rate of elimination.

Pharmacology is the study of the action of drugs on the lower animals, by which much can be learned as to the adaptability of drugs in therapy and their mode of action, their advantages and disadvantages in comparison with others of similar character.

Pharmacological assaying is the application of this information so that powerful drugs can be standardized although possessing no chemical reactions by which their values can be determined.

The scope of each is distinctly defined, the assay process being subordinate to and based entirely on the pharmacology of the drug in question.

The criticisms of the biologic assay process in general are really without material basis since it is strictly analogous to the chemical assay. Neither assay method attempts to encroach on the clinical application of a drug, but concerns itself only with the potency. Both make use of the reaction which most accurately determines the amount of active agent present by use in the one case of chemical reagents, in the other of the animal least subject to variations.

Digitalis, therefore, as one of the most valuable drugs at the command of the physician, cannot be permitted to pass without standardization.

The selection of the method should be based on the most typical effect which is measurable and which is subject to the least variation that is beyond the control of the operator.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 261, 1920.

(Reprinted from the *Journal of the American Veterinary Medical Association*, Vol. LVII, N. S. Vol. 10, No. 6, September, 1920.)

STUDIES ON ANTHELMINTICS.

X. Stock Tonics and Some of Their Constituents.

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One of us (Hall, 1917, 1918, 1919) has stated elsewhere the belief that the mineral mixtures or stock tonics commonly employed are of very little value as anthelmintics. This seems to us necessarily true. Potent anthelmintics are too toxic and dangerous to put into these preparations in sufficient amounts to be effective, because these preparations are used by persons unskilled in the handling of potent drugs. Only innocuous substances, usually reputed to be anthelmintic, and often with but little besides the reputation of being anthlemintic, can safely be incorporated in these preparations. Of the feeble anthelmintics used, iron sulphate and common table salt are the favorites. The remainder of the preparations consists for the most part of purgatives, tonics, antacids, flavor and color. We give below some tests in support of the idea that neither the mineral mixtures, stock tonics, nor the iron sulphate and salt that make up a large part of their bulk are dependably anthelmintic. These tests show that these things are not valuable when administered carefully as drugs; they will be even less effective when given in feed to stock, and there would be no point to these preparations if they could not be given in feed, as they are essentially designed for this to save the trouble of dosing.

The following experiments with commercial preparations have been briefly abstracted by Hall (1919):

Commercial preparation No. 1, a well-known preparation, was given to dog No. 232, an animal weighing 10 kilos, as follows: The dog received 6 teaspoonfuls of the preparation daily—about half of the dose for a hog—for a total of 14 doses in 16 days, or 84 teaspoonfuls. The dog passed 2 ascarids, and had 10 ascarids

and 93 *Dipylidium* post-mortem. The treatment was, therefore, about 17 per cent effective against ascarids and 0 per cent effective against tapeworms. At this rate the ascarids would have been removed in 3 months, assuming that it removed 1 worm a week. This is an iron sulphate and sodium chloride preparation.

Commercial preparation No. 2 was given to dog No. 327, an animal weighing 10 kilos, as follows: The dog was first given 1 teaspoonful a day, the dose for a 100-pound hog, but as this caused vomiting, owing to the salt, which makes up 95 per cent of the preparation, the dose was cut to ½ teaspoonful, for a total of 10 teaspoonfuls in 19 days. The dog passed no worms and had 1 ascarid post-mortem. Treatment was therefore 0 per cent effective.

Commercial preparation No. 3 was given to dog No. 298, an animal weighing 14.5 kilos, at the indicated rate of 1 teaspoonful daily, for a total of 27 doses in 32 days. The dog passed 1 ascarid (after 8 treatments) and on post-mortem had 15 ascarids and 18 tapeworms. The treatment was therefore about 6 per cent effective against ascarids and 0 per cent effective against tapeworms.

These experiments show that the stock tonic group has but little anthelmintic value.

To determine the anthelmintic efficacy of salt and iron sulphate the following tests were made:

Common salt was given in a dose of 1 gram in capsule followed by a small amount of water, about a half-ounce, to dog No. 325, weighing 8 kilos. The dog passed no worms and was found on post-mortem to have 2 hookworms, 4 whipworms and 3 tapeworms. Treatment was 0 per cent effective against hookworms, whipworms and tapeworms.

Iron sulphate was given to 4 dogs as follows:

Dog No. 37, weighing 2 kilos, was given a 5-grain dose on each of 2 successive days, the first dose accompanied by 5 grains of calomel. The dog passed 1 ascarid and 8 whipworms, and on post-mortem had 5 ascarids and 100 tapeworms. The treatment was therefore about 17 per cent effective against ascarids, 100 per cent effective against whipworms, and 0 per cent effective against tapeworms.

Dog No. 15, weighing 8 kilos, was given the same dose, 5 grains, on 4 successive days, accompanied by 2 grains of calomel on the

second day. The dog passed no worms and had 1 whipworm post-mortem. Treatment was 0 per cent effective against whipworms. There might have been more whipworms; this dog died from an intussusception of the ileum through the ileo-colic valve into the colon, and the specimen was kept intact.

Dog No. 44, weighing 10 kilos, was given 5 grains daily for a total of 13 doses in 18 days, or 65 grains. The dog passed no worms, and on post-mortem had 20 whipworms and 11 tapeworms. Treatment 0 per cent effective against whipworms and tapeworms.

Dog No. 242, weighing 16 kilos, was given iron sulphate in doses beginning at 5 grains the first day and increasing by 5 additional grains daily to a dose of 40 grains. The dog received 8 doses in 9 days, a total of 3 drams and an average dose of 32.5 grains. The dog passed 1 ascarid, and 1 more was found in the large intestine post-mortem, and must be accredited to the anthelmintic. It also passed 3 whipworms. Post-mortem there were 674 whipworms. The treatment was therefore 100 per cent effective against ascarids and less than 0.5 per cent against whipworms.

From the foregoing we may note that: Salt in the dose given is apparently of no value against hookworms, whipworms and tapeworms, but it would need further experiment to determine just how little anthelmintic value it has. Iron sulphate must be given in very large doses to be really effective against ascarids in the dog. It is of interest to recall that this drug is commonly employed against ascarids in the horse, much more difficult worms to remove. Iron sulphate shows itself effective against whipworms occasionally, but is evidently not dependable, as the results with the large doses given dog No. 242 show. It has no value for removing tapeworms. The commercial preparations—and this is true for other mineral mixtures, as one of us (Hall) has found in tests—are of but little value as anthelmintics. They may have value as appetizers or to supply mineral constituents.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 262, 1920.

(Reprinted from *Journal of Bacteriology*, Vol. V, No. 5, September, 1920.)

BACTERIOLOGIC PEPTONE IN RELATION TO THE PRODUCTION OF DIPHTHERIA TOXIN AND ANTITOXIN.

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I. INTRODUCTION.

The necessity of using peptone media in the production of potent diphtheria toxin was recognized by the pioneer investigators in this field of serum therapy. Park and Williams (1896) appear to have been the first to lay any special emphasis on this constituent of the culture medium. After experimenting with varying concentrations of peptone in diphtheria toxin broth, they conclude that the strength of toxin averages greater with higher percentages of peptone (2 and 4 per cent) than with lower percentages (1 per cent). This is corroborated by Theobald Smith (1899), in his publication on the relation of glucose to the production of toxin in bouillon cultures of the diphtheria bacillus. He also proposed the use of a peptone bouillon in which the beef infusion, after preliminary reduction in acidity, was submitted to a fermentation with *B. coli*, in order to remove muscle sugar. Previous investigations by Spronck and von Fourenhout (1895) had indicated an inhibitory action of this carbohydrate upon the elaboration of toxin in peptone bouillon cultures of *B. diphtheriæ*.

Martin (1898) attributed unsatisfactory results in toxin production to variations in composition of the peptone then available which appears to have been the Witte product. To overcome this, he proposed what he terms "liquid peptone" obtained from auto-digested swine stomachs which he mixed with an equal volume of veal bouillon. As would be expected, from its composition, this medium did not give successful results in the hands of other investigators, and found little application in practice.

Spronck (1898) in the same year recommended the substitution of a boiled and filtered extract of commercial yeast in place of the infusion of veal or beef. However, he specifically required the use of Witte peptone with his yeast product.

Subsequent publications on media for diphtheria toxin production appear to be more concerned with the reaction of the broth, and also with the kind and condition of the meat used in the infusion. Veal is recommended by some in place of beef, while others insist that the meat before using must be either decomposed or fermented instead of freshly killed. This is in conformity with Theobald Smith's proposal for eliminating muscle sugar, which is assumed to interfere with toxin elaboration. Aside from the mention that the Witte product was employed, no attention appears to have been paid to peptone by later investigators.

The scarcity of Witte peptone during the past few years has again directed attention to bacteriologic peptone and to the methods of producing diphtheria toxin. A number of peptone products have appeared, of differing composition, which, while allowing growth of the more common microörganisms and of *B. diphtheriæ*, do not permit of obtaining the strong toxins formerly obtained. The use of trypsinized media as suggested by Cole and Onslow (1916) and the various modifications of Martin's peptone solution which have been recommended have not fulfilled practical requirements.

In the attempt to explain the necessary conditions for the successful application of some of the substitute products, rather unique views have been advanced concerning diphtheria toxin formation. Bunker (1919) in a recent article suggests "that there is a point at which toxin development is at its maximum, before which and after which the potency will be lost." He also states that "if time alone is made the basis of judging when toxin is 'ripe,' it will be only by chance and in spite of technique that any peptone will give satisfactory results." These statements, as will be later shown, are contrary to extended practical experience in the routine production of high potency diphtheria toxin.

During the past year, the writer has had an opportunity to study large scale manufacture of diphtheria toxin. This included the preparation of thousands of liters of toxin having an L^+ dose of 0.33 cc., 0.25 cc., or less, and its use in horses for immunization

purposes. It is the purpose of this article to consider the essentials for the routine production of high potency diphtheria toxin with special reference to its application in the development of high strength antidiphtheric serum.

II. THE PRODUCTION OF DIPHTHERIA TOXIN.

a. Culture to be employed.

Practically all of the institutions engaged in the propagation of *B. diphtheriæ* for toxin elaboration employ the strain known as "Park-Williams bacillus no. 8" or "Culture Americana." Recently, six cultures of this strain were obtained from as many different laboratories. All were similarly carried by transplanting from two twenty-four-hour generations on Loeffler slant tubes and then in bouillon, according to technique later described. A decided variation in the strength of the final toxin, from the six cultures, was noted; two gave toxin of which one L^+ dose was greater than 0.5 cc. Only one, from the Hygienic Laboratory of the United States Public Health Service, gave toxin having an L^+ dose of 0.25 cc. or less. The toxins from two of the remaining three cultures gave L^+ doses of 0.50 cc. and the other one had a strength of $L^+ = 0.33$ cc. The advisability of verifying the toxicogenicity, particularly of any new strain of the organism, is apparent. The parent culture is best maintained on slant tubes of moist Loeffler blood serum, transplants to bouillon being made as desired.

b. Culture medium.

Of the various media recommended for toxin elaboration with *B. diphtheriæ*, we have found plain beef infusion broth containing 2 per cent peptone and 0.5 per cent sodium chloride to be the most satisfactory. Extended trial with veal infusion in place of the beef has not demonstrated any increase in toxicogenicity or shown any other advantages to warrant its use. Beef from various sources, including both cold storage and fresh products, has been employed in the infusion with equally satisfactory results. The increased acidity at times encountered with cold storage meat has not been found to require any special treatment. Preliminary fermentation of the infusion with a culture of *B. coli*, as first recommended by Smith (1899), to remove any fer-

mentable carbohydrates is, at best, an unsatisfactory procedure, and in our experience entirely superfluous. The formation, by the colon bacillus, of decomposition products which may give trouble on injection of the final diphtheria toxin, must also be taken into consideration.

Allowing the beef to infuse over night is not very practical with large scale preparation. Equally satisfactory results are obtained by adding twice the quantity of water to the minced beef and bringing to a boil in the steam kettle in the course of about an hour and a half. The resultant infusion liquor is then obtained by the use of a suitable press.

Fat must be eliminated from beef infusion, as even traces of fat have a decided inhibitory action on the production of diphtheria toxin. This has been demonstrated on numerous occasions when, out of the same lot of broth, those flasks which showed particles of fat gave final toxin of which the L^+ dose was greater than 0.5 cc., while the strength in the remaining flasks was 0.25 cc. or less. We are probably dealing here with a surface tension phenomenon, similar to that discussed by Larson, Cantwell and Hartzell (1919) in their recent paper on the influence of the surface tension of media on the growth of bacteria. It seems quite likely that the fat depresses the surface tension of the bouillon, thus forcing *B. diphtheriæ* to grow beneath the surface with resultant diminution in the formation of pellicle and toxin.

Experimentation with differing concentrations of peptone from 0.5 per cent to 4 per cent has shown that the most potent toxin requires a peptone content around 2 per cent. Amounts up to 4 per cent may be satisfactorily employed, but with no advantage over the smaller concentrations. The peptone used in preparing the toxin under observation was Bacteriologic Peptone, Parke, Davis & Company, the composition and properties of which have been described in a previous article (Davis, 1917). Twenty grams of peptone and 5 grams of sodium chloride were added to every liter of beef infusion prepared as above, dissolved in the cold and then brought to a boil in the steam kettle to insure thorough solution.

Considerable uncertainty appears to exist as to what is the most satisfactory initial reaction for diphtheria toxin bouillon. Nearly all of the previous investigators, including Roux and Yersin

(1888), Spronck (1898), Madsen (1897), Park and Williams (1896), Smith (1899) and Lubenau (1908), have employed either neutralization with litmus, which is crude at best, or "hot titration" with phenolphthalein, a procedure which is admittedly fallacious. The writer (Davis, 1918) in a paper on "Hydrogen ion concentration and toxicogenicity determinations with *Bact. diphtheriæ*," has shown that potent toxin is produced in bouillon by *B. diphtheriæ* only when the initial reaction falls within a certain zone of alkalinity, included within the hydrogen ion concentration limits of about 7.0×10^{-8} (pH = 7.2) to about 5.0×10^{-9} (pH = 8.3). The maximum degree of potency, however, is obtained when the reaction of the broth comes within the narrow limits of pH = 8.0 to pH = 8.2. This may be readily and consistently obtained by following the procedure given below for adjusting the reaction.

1. Transfer 10 cc. of the heated broth to a small Erlenmeyer flask and dilute with about 40 cc. of cold, distilled water. Add 0.5 cc. of a 1 per cent solution (95 per cent alcohol) of phenolphthalein as indicator and titrate to a deep pink color against an N/10 NaOH solution. The latter is preferably prepared when required as an exact 1/100 dilution of a 10/N stock solution. The amount of the strong (10/N) solution required to neutralize per liter of medium is given directly by the burette reading. Bring to a boil again in the steam kettle, and estimate the hydrogen ion concentration.

2. While the more accurate hydrogen electrode method is desirable for comparative and standardization purposes, equally satisfactory results for routine production may be obtained by the colorimetric method. The simple "comparator" of Hurwitz, Meyer, and Ostenberg (1916) is recommended with standardized boric acid-potassium chloride-sodium hydroxide mixtures of pH = 8.0 and pH = 8.2, prepared as directed by Clark and Lubs. Exactly 10 cc. of the neutralized bouillon are transferred to a "comparison" tube, diluted with 10 cc. of distilled water, and mixed well. Ten cubic centimeters of the mixture are now removed to another tube and 0.5 cc. of an 0.02 per cent solution of phenolsulphonephthalein in 50 per cent alcohol next added. Two other tubes are prepared containing 10 cc. respectively of the standardized pH = 8.0 and pH = 8.2 mixtures, with 0.5 cc. of the phenol-

sulphonephthalein solution in each tube. The technique for comparison is as given by Clark and Lubs (1917). Usually, the value will be found to very closely approximate pH = 8.2. If, as may sometimes happen, the color in the tube containing medium plus indicator is lighter than the compensated pH = 8.0 standard, 10/N NaOH can be added directly to the former tube until the desired tint is reached. The burette reading, multiplied by two (since the equivalent of 5 cc. of the medium is used) gives the amount of 10/N NaOH required to correct each liter of broth.

Experience has repeatedly confirmed the observation that toxin of greater potency is obtained from broth contained in large flasks than in small ones. Other conditions being equal, we can expect to find diphtheria toxin of higher strength after growth in a six-liter flask containing three liters of broth than in a liter flask containing 500 cc. Three liters of broth, dispensed into 6-liter, Florence type flasks have been employed in producing the toxin under observation.

It has undoubtedly been noted in every laboratory engaged in diphtheria toxin production that, if for some reason it becomes necessary to resterilize media, there is a resultant diminution in the strength of the final toxin. From experimentation in progress, to be reported upon in a later publication, it appears that food accessory factors, possibly of a vitamin character, are concerned in the production of diphtheria toxin. This makes it especially desirable that the sterilization period of the medium should be as short as possible, to reduce destruction of the accessory factors to a minimum and yet be sufficient to ensure thorough heat penetration. Autoclave sterilization at 120°C. (15 pounds steam pressure) for a period not exceeding thirty minutes has given satisfaction.

c. Cultivation.

The parent culture of *B. diphtheriæ* on the Loeffler slants is transplanted through several twenty-four-hour generations in tubes containing 10 cc. of bouillon to stimulate maximum pellicle formation. The tubes are then used to inoculate small "starter" flasks containing 30 cc. of medium, which are also incubated for twenty-four hours. The large flasks of broth are now inoculated with the twenty-four-hour "starters," allowing one for each large flask. A temperature range of 36° to 38°C. has been

found most satisfactory for incubation. Numerous potency tests have demonstrated that at least ten days' incubation of the large flasks is necessary to ensure maximum elaboration of toxin, and a twelve-day period is desirable. As has been shown in a previous publication (Davis, 1918), toxin of appreciable strength is elaborated by toxicogenic cultures within forty-eight hours. The potency gradually increases to a maximum value about the twelfth day, occasionally sooner. Incubation for an additional period of two weeks, or four weeks altogether, shows no deterioration of the final toxin. That this behavior is not confined specifically to the peptone employed is proved by the fact that, with the procedure as given above, Witte's peptone permits of similar results. In this case, toxin of maximum potency is obtained in the large flasks, only after a two-weeks' incubation.

When cultivated in plain bouillon under the optimal conditions already described, *B. diphtheriæ* causes an initial increase in the hydrogen ion concentration of the medium. This is soon followed by a steady decrease until, apparently, a limiting alkaline reaction is attained. The following table, taken from the article on hydrogen ion concentration determinations mentioned above (Davis, 1918), shows these changes with a toxicogenic strain.

Changes in H-ion concentration and toxicogenicity during growth of B. diphtheriæ in bouillon.

TIME	C_H	pH	TOXICITY L^+ DOSE
hours			*cc.*
0	7.0×10^{-9}	8.15	
24	2.1×10^{-8}	7.68	1.0
48	3.2×10^{-8}	7.49	0.8
72	3.8×10^{-8}	7.41	
96	3.4×10^{-8}	7.47	0.55
120	2.1×10^{-8}	7.68	
144	2.5×10^{-8}	7.61	0.45
192	2.3×10^{-8}	7.64	
240	1.6×10^{-8}	7.79	0.15
312	8.7×10^{-9}	8.05	0.15
408	7.0×10^{-9}	8.15	0.15
528	5.0×10^{-9}	8.30	0.15

It is further shown in the same publication that the final hydrogen ion concentration of high strength toxin L^+ dose (less than

0.25 cc.) after two weeks incubation ranged from $C_H = 1.6 \times 10^{-8}$ (pH = 7.79) to $C_H = 5.2 \times 10^{-9}$ ($pH^+ = 8.28$). At the same time, low strength toxins were obtained, the final H ion concentration of which came within the above limits. It is obvious from the table that in the normal development of *B. diphtheriæ* in bouillon, the organisms may produce the same H ion concentration at two different intervals which represent wide variations in potency. This fact, and what has been stated above, justify the conclusion that there is no direct relationship, during or after growth, between the H ion concentration of the medium and production of toxin.

d. Final operations.

The contents of the large toxin flasks, after proper incubation, are checked microscopically to determine purity of culture; 0.4 per cent of purified cresols is then added and allowed to act for, at least, twenty-four hours to ensure thorough germicidal action. As a rule, filtration can be accomplished satisfactorily through paper, otherwise Mandler filters may be employed. During the above operations and in the finished condition, the toxin should be stored in a cool place.

It has been our experience in evaluating the strength of the diphtheria toxin for injection purposes, that the L^+ dose method (according to the Hygienic Laboratory) furnishes a more reliable index than determination of the minimum fatal dose. Although the theoretical relationship may not exactly hold, the L^+ dose for all practical purposes may be considered as 100 M. F. D.

III. GENERAL OBSERVATIONS.

It will be readily conceded that we can best judge the value of what has been presented from the actual results of practical application. Data obtained during the past year in the production of several thousand gallons of diphtheria toxin by the general method indicated, are summarized below.

Diphtheria toxin

Percentage of total toxin having L^+ dose

ABOVE 0.50 CC. (M. F. D. > 0.005 CC.)	0.50 CC. (M. F. D., 0.005 CC.)	0.33 CC. (M. F. D., 0.0033 CC.)	0.25 CC. OR LESS (M. F. D., 0.0025 CC. OR LESS)
10.2	11.3	35.5	43.0

PRODUCTION RESULTS.

As may be noted, practically 90 per cent of the toxin produced was of usable strength, —L^+ dose = 0.50 cc. or less. Of this, more than 78 per cent was high strength, having an L^+ dose of 0.33 cc. or less, and 43 per cent was so strong that one L^+ dose was 0.25 cc. or less. In view of the fact that only about 10 per cent of the large amount of toxin produced failed to reach a desirable strength, the procedure and medium recommended above can be considered as meeting practical requirements.

It is interesting to note, in connection with the production of diphtheria toxin, that no definite seasonal or weekly variation, as mentioned by MacConkey (1912), was observed. Occasionally, one or two bottles of a large lot would show an inferior or weak toxin, in spite of the fact that, as far as could be determined, the contents of all bottles after filtration should have been identical.

Data have been obtained in this study supporting the view that the troublesome local reactions encountered with horses in diphtheria treatment may be largely attributed to the method of controlling the hydrogen ion concentration of the toxin bouillon. It is a fact that in the use of the toxin under observation, practically no local reactions have been experienced. It is also true that adjusting the toxin bouillon by the colorimetric H ion method discussed above requires considerably less alkali than the use of the inaccurate "hot titration" method formerly employed. Whether it is the decreased amount of alkali used or possibly a diminished content of toxone bodies in the final toxin which is responsible for the favorable results must be left for further study.

Consideration of the foregoing production results would not be complete without data showing the antitoxic response to injection of the diphtheria toxin in horses. In the final analysis, this determines the utility of the toxin and, consequently, the value of the methods and culture medium which are recommended. A summary has been prepared in the succeeding table to show the potency of the antidiphtheric serum obtained in the first yield from horses immunized during the past year with the toxin under discussion. The first large scale bleeding has been chosen for the valuation because experience has shown that the potency of this serum represents more closely than that from any of the succeeding bleedings the true value of the toxin injected.

Diphtheria antitoxin

Percentage of new horses yielding serum

UP TO 200 A. U.	200 A. U. TO 500 A. U.	500 A. U. TO 1000 A. U.	1000 A. U. TO ABOVE 1500 A. U.
22.4	27.7	34.3	15.6

Analysis of the table shows that nearly 78 per cent of all the new horses injected during the past year with the toxin under consideration produced a serviceable antidiphtheric serum (*i. e.*, having a potency on first bleeding of 200 antitoxin units or greater). Eighty per cent of the productive horses (or 82 per cent of the total number) gave serum ranging from 200 to 1000 units, 20 per cent (15.6 per cent of the total number) yielded the high potency products from 1000 to above 1500 antitoxin units, and 44 per cent (34.3 of all of the treated horses) came within the moderately high range from 500 to 1000 a.u. The foregoing, and the fact that it has been possible in routine operation to immunize horses to an antitoxin strength exceeding 1500 units per cubic centimeter, can be taken as proof that the toxin used is fully satisfactory to meet all requirements of diphtheria antitoxin production.

SUMMARY.

1. A résumé of the more important literature on the production of diphtheria toxin is given. This shows wide divergence of procedure. The recent scarcity of Witte peptone and failure of many substitute products to allow of appreciable toxin formation have further complicated the methods employed.

2. The essentials for the routine production of high potency diphtheria toxin are discussed. It is shown that, other conditions being the same, the toxicogenicity of the culture employed may vary within wide limits, according to the source. The necessity of verifying toxin production with any new strain is made apparent.

3. Plain beef infusion bouillon, containing peptone and salt, is recommended for toxin production. Preliminary fermentation of the infusion with a culture of *B. coli* is shown to be undesirable. The use of veal infusion in place of beef is unnecessary.

Even traces of fat must be avoided in the infusion as it interferes with maximum pellicle formation and thus diminishes toxin elaboration.

4. A content of 2 per cent peptone with 0.5 per cent of salt in the bouillon has been found to be most satisfactory.

5. Maximum strength of the final toxin has been obtained when the reaction of the broth comes within the limits of $pH^+ = 8.0$ to $pH^+ = 8.2$. A procedure is given for adjusting the hydrogen ion concentration to these values.

6. Cultivation for toxin production is best made in large flasks, previously inoculated with twenty-four-hour cultures in small "starter" flasks. Incubation is for at least ten days at 36°–38° C., with a twelve-day period preferred. Data are presented showing that the H ion concentration of the medium during growth cannot be used as an index of diphtheria toxin production.

7. Results are tabulated which have been obtained during the past year in the production of several thousand gallons of diphtheria toxin according to the procedure discussed. Practically 90 per cent of this toxin was of serviceable strength, —L^+ dose = 0.50 cc. or less. Of this more than 78 per cent was high strength, having an L^+ dose of 0.33 cc. (M. F. D. = 0.0033 cc.) or less, and 43 per cent had an L^+ dose of 0.25 cc. (M. F. D. = 0.0025 cc.) or less.

The efficiency of this toxin in the routine immunization of horses for antitoxin is shown by records of antidiphtheric serum production during the past year. Nearly 78 per cent of all new horses on this treatment produced serviceable antidiphtheric serum, *i. e.*, having a potency on the first large scale bleeding of 200 antitoxin units or greater. Of the productive horses, 80 per cent gave serum ranging from 200–1000 units, 44 per cent yielded a product from 500–1000 units, and 20 per cent had serum coming within the very high range from 1000 to above 1600 antitoxic units per cubic centimeter.

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Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 263, 1919.

NOTES ON THE MICHIGAN FLORA. II.

(Reprinted from the Twenty-first Michigan Acad. Sci. Rept., 1919.)

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In the Michigan Flora[1] the enumeration begins with the Ophioglossaceae, presumably under the supposition that this family is the most primitive of the Filicales. In these notes the order of genera is that of Gray's Manual, ed. 7.[2]

POLYPODIACEAE.

Phegopteris Robertiana (Hoffm.) A. Br. is to be looked for in Michigan as its range crosses the State.

Allosorus and Cheilanthes were published in the same year, the former appearing first. It was based on the "Adianta spuria" of Swartz, some nine species, eight of which were included in Cheilanthes by Swartz, who referred Allosorus as a synonym to his Cheilanthes. Since Allosorus was based not on one species but on all the "Adianta spuria" (nine species) of Swartz, the name must therefore be retained for that group containing the larger number of species which are congeneric. Also according to the Vienna Code, Article 45, "If . . . . one of the parts detached contains a great many more species than the others, the name is reserved for that part of it." Of the nine species on which Bernhardi founded Allosorus, Swartz referred eight to his Cheilanthes, creating a new genus, Mohria, for the other. The majority of the species on which Bernhardi founded Allosorus are unquestionably congeneric with those species on which Swartz founded his Cheilanthes, and the two

[1]Beal, W. J., Michigan Flora. A list of the Ferns and Seed Plants growing without cultivation. (Reprinted from Fifth Report of the Michigan Academy of Science, 1904.) Lansing, 1904.

[2]In the region of Gray's Manual there are three other species of the genus. These are *Allosorus Alabamensis* (Buckley) O. K.; *Allosorus tomentosus* (Link) n. comb. (*Cheilanthes tomentosa* Link, Hort. Berol. 2, 42, 1833); *Allosorus gracilis* (Fee) n. comb. (*Myriopteris gracilis* Fee, Gen. Fil. 150, 1850-2). The earlier *A. gracilis* of Presl. is a synonym of *Cryptogramma Stelleri* (Gmel.) Prantl. and cannot interfere with the use of the name "gracilis" for this species.

generic names are synonymous. Art. 46, "When two or more groups of the same nature are united, the name of the oldest is retained." As Allosorus is older than Cheilanthes the latter must give way to the former. This may be deplorable, but no other conclusion can be arrived at under the Vienna Rules.

Allosorus lanosa (Mx.) n. comb. (*Nephrodium lanosum* Mx. Fl. Bor. Am., 270, 1803). The range of this species crosses Michigan; it should be looked for in the rocky sections of the Upper Peninsula.

I have been informed by Dr. H. T. Darlington of the Michigan Agricultural College that the specimens of *Pellaea atropurpurea* (L.) Link from Michigan have a glabrous rhachis and rachiola which proves our plant to be *P. glabella* Mett.

Asplenium viride Huds. should be looked for in the lime-stone regions of the Upper Peninsula.

The proper authority for the combination *A. Filix-femina* var. *Michauxii* (Spreng.) is Clute.

Woodsia scopulina D. C. Eaton of Michigan and Minnesota has been described as a new species, *W. Cathcartiana Robinson;* the former name should therefore disappear from the Michigan Flora. *W. glabella* R. Br. should be looked for along the shores of Lake Superior.

Pteretis is the oldest name for the Ostrich Fern, as shown by Dr. Nieuwland. Our plant is not exactly identical with the European type. It has been described as a distinct species and as a variety. The differences, however, are scarcely of specific value and it may better be considered as a good geographical variety. It may be known as Pteretis Struthiopteris (L.) Nieuwl. var. Pennsylvanica (Willd.) n. comb. (*Struthiopteris Pennsylvanica* Willd. Sp. Pl. v. 289, 1810).

Osmunda cinnamomea, L. var. *frondosa Gray.* This rare form with some of the fertile fronds partly sterile below was found at Port Huron, June 23, 1918. No. 4,960.

Osmunda regalis L. var. *spectabilis* (Willd.) Milde. The specific type has green fronds at all times and the pinnules are abruptly narrowed just above the broad base, thence gradually tapering to an apex. I have not found anything answering to this form. Our plant is purplish when young, often glaucous on the stipes and the pinnules are not contracted just above the

base. It answers to Willdenow's *O. spectabilis* and is a good geographical variety.

OPHIOGLOSSACEAE.

The plant listed as *Botrychium boreale* (Fries) Milde is a form of *B. Matricariaefolium* R. Br.

EQUISETACEAE.

The evergreen species of Equisetum (Tourn.) L. have been taken out of that genus and now constitute the genus Hippochaete Milde. For species and varieties see *Memoirs New York Botanical Gardens*, Vol. 6, pages 161-72, and *American Fern Journal*, Vol. 7, pages 73-6, 1917. *H. prealta* var. *intermedia* was collected near Port Huron, June 23, 1918, No. 4,962. *H. Nelsoni* was collected in Lenawee County, in 1917, by Mr. Cecil Billington.

SELAGINELLACEAE.

Fernald has shown that "apus" is not the Linnaean specific appellation for our slender, creeping species. The proper binomial is *Selaginella apodum* (L.) Fernald.

PINACEAE.

P. Banksiana Lamb. is the proper designation for the Jack Pine.

Two species, the White spruce, *Picea Canadensis* (Mill.) B. S. P., and the Hemlock Spruce, *Tsuga Canadensis* (L) Carr. derive their specific names from the same source—the *Pinus Canadensis* L. It is true that "(Mill.)" is quoted as the original author of the specific name of the former, but this is an error, as Miller took his specific name and technical description bodily from Linnaeus. One or the other must drop the specific name Canadensis, as the specific name can apply to but one element of the aggregate when it is segregated. The White Spruce is *Picea Canadensis* (L.) B. S. P.; the Hemlock Spruce is *Tsuga Americana* (Mill.) Farwell. See *Bul. Tor. Bot.* Cl. Vol. 41, page 621, 1914, and *Rhodora*, Vol. 17, pages 164-8, 1917.

Juniperus Virginiana L. On the rocky shores of Keweenaw Point, many years ago, I collected specimens of a prostrate Juniper, creeping or trailing over rocks, with the habit of *J. horizontalis;* the leaves and fruit on erect peduncles proved it to be *J. Virginiana* L. I have not seen the trailing form since that time.

Juniperus Sabina L. The American Savin is now generally considered to be a distinct species from the European and should be known as *J. horizontalis* Moench.

TYPHACEAE.

Typha latifolia L. The common Cat-tail is found throughout the State along the borders of streams and ponds. The typical form has both the staminate and pistillate spikes of about equal length and without any space between them. A form with the spikes about 3 centimeters apart is var. *ambigua* Sond. Shores of Belle Isle, Aug. 11, 1893; No. 353b. Another form with the spikes slightly separated and the staminate spike longer than the pistillate is the var. *remotiuscula* Simonkai. Keweenaw Co., September 4, 1885, No. 353; Ypsilanti, June 21, 1892, No. 383a.

Typha angustifolia, L. The narrow-leaved Cat-tail is usually said to be rare inland. I have seen acres of it in marsh lands in the vicinity of Wind Mill Point at the head of Detroit River, Sept. 19, 1901, No. 1,761; Oakwood, Sept. 23, 1915, No. 4,108; Junior, July 13, 1918, No. 5,076.

SPARGANIACEAE.

Sparganium simplex var. angustifolium is now considered as specifically distinct and should be known as *S. angustifolium* Mx.

FLUVIALACEAE.

Potamogeton diversifolius Raf. of the Michigan Flora should become *P. hybridus* Mx.

Potamogeton filiformis Pers. should become *P. marinus* L. Among many modern botanists there has sprung up a very disconcerting practice, and a needless one as well, of replacing old and well known names by those of a later date, because, forsooth, the authors are not able to interpret to their own satisfaction the older descriptions; hence the name is set aside as a "confusing" name. There would be much less "confusion" if old names were not discarded unless it could be proved beyond a shadow of doubt to what species they do belong; this cannot be done. To retain the old name, therefore, for the species for which it had stood unchallenged for perhaps a century and a half, more or less, would be not only the better plan but much less "confusing."

Potamogeton interruptus Kit. should be dropped as it has been shown by Mr. St. John that this species, if distinct from *P. pectinatus* L., does not occur in this country.

P. lonchites Tuckerman becomes *P. Americanus* C. & S.

P. Nuttallii Cham. and Sch. becomes *P. epihydrus* Raf.

P. spathulaeformis (Robbins) Morong. becomes *P. spathaeformis* Tuckerman.

P. Spirillus Tuckerman becomes *P. dimorphus* Raf.

P. Zizii Roth becomes *P. angustifolius* Berch. & Presl.

P. zosteraefolius Schum. becomes *P. compressus* L.

P. Friesii Ruprecht becomes *P. mucronatus* Schrad.

Other species and varieties credited to Michigan are the following:

P. epihydrus var. *Cayugensis* (Wieg.) Benn.

P. Americanus var. *Novaeboracensis* (Morong.) Benn.

P. bupleuroides Fernald.

P. strictifolius Benn.

P. rutilus Wolfg. In various slips at Detroit June 29, 1900, No. 1,636a.

NAJADACEAE.

Najas flexilis (Willd.) Rost. & Schm. var. CONGESTA n. var. Stems short, much branched, and forming a dense, more or less globular mass, like a small pin cushion, 1-3 inches in diameter. Detroit R.; Sept. 23, 1892, No. 1,312b.

"Naias" should be spelled with a "j," not with an "i."

JUNCAGINACEAE.

Scheuchzeriaceae should become Juncaginaceae.

ALISMOIDACEAE.

Alismaceae should become Alismoidaceae.

Sagittaria sagittaefolia L. becomes *S. latifolia Willd.;* the forma *angustifolia* becomes *S. latifolia f. gracilis* (Pursh.) Robinson; the other forms enumerated remain, but the authority for them under *S. latifolia* is Robinson instead of Britton. *S. rigida* Pursh becomes *S. heterophylla* Pursh with vars. *elliptica* Engelm; *rigida* (Pursh) Engelm; and *angustifolia* Engelm.

Echinodorus tenellus (Mart.) Buch. This is no longer considered to be a species of Alisma.

A. Plantago-aquatica L. is not found in America; there is a variety *Americana* R. & S. and a variety *parviflora* (Pursh) Farwell. Both are found in Michigan.

HYDROCHARIDACEAE.

Valisneriaceae becomes Hydrocharidaceae.

Philotria Raf. becomes Elodea Mx.

P. Canadensis (Mx.) Britton becomes *E. Canadensis* Mx.

GRAMINACEAE.

Gramineae becomes Graminaceae.

Zea Mays L. Corn, Maize. Adventitious.

Andropogon scoparius Mx. var. *frequens Hubb.* replaces *Andropogon scoparius* Mx.

Sorghastrum nutans (L.) Nash should replace *S. avenaceum* (Mx.) Nash.

Syntherisma Walt. becomes Digitaria Heist.

S. linearis (Krock) Nash. becomes *D. linearis* (L.) Pers.

S. sanguinalis (L.) Dulac. becomes *D. sanguinalis* (L.) Scop.

D. filiformis (L.) Koeler is credited to Mich. in Gray's Manual.

Echinochloa Crusgalli (L.) Beauv. Greenish or yellowish with awns up to about 2 cm. in length. Keweenaw Co., No. 648, Aug. 8, 1888; Detroit, No. 648b, July 21, 1892.

E. CRUSGALLI forma MITIS (Pursh) n. comb. (*Panicum Crusgalli* var. *mite* Pursh. Fl. Am. Sept. 1, 66, 1814). Most of the flowers are muticous, not awned. Detroit, Aug. 18, 1893, No. 648a; Orion, Aug. 29, 1895, No. 648c; Marquette, Aug. 1902, No. 648d.

E. CRUSGALLI forma PURPUREA (Pursh) n. comb. (*Panicum Crusgalli* var. *purpureum* Pursh. Fl. Amer. Sept. 1, 66, 1914.) Panicles purplish, flowers muticus to acuminate, the acumination about as long as the floret. Detroit, Oct. 2, 1900, No. 1701.

E. Crusgalli (L.) Beauv. forma SABULONUM n. f. (*Panicum Crusgalli* var. *sabulonum* Trin. Gram. 2, pl. 163, 1829). Panicle pyramidal, lower branches elongated (10 cm. long), with secondary branchlets 2-3 cm. in length, awns 3 cm. long, purplish and hispid. Detroit, Oct. 2, 1900, No. 1701½.

E. Crusgalli (L.) Beauv. forma *longiseta* n. f. (*Panicum*

Crusgalli var. *longisetum* Trin. Gram. 2, pl. 162, 1829?). Similar to the species but awns about twice as long (up to 5 cm.) and more or less purplish; bulbous bases of hairs slightly larger and more numerous; appearing as if indigenous. Marshy borders of Belle Isle, Sept. 23, 1904, No. 1887.

E. CRUSGALLI var. MURICATA (Mx.) n. comb. (*Panicum muricatum* Mx. Fl. Bor. Am. I, 47, 1803; *E. muricata* (Mx.) Fernald). This variety is rare compared with the preceding. The rigid hairs and their bulbous bases are conspicuous and give to the spikelets somewhat the appearance of a Cenchrus. Detroit, Aug. 13, 1902, No. 1782.

Cenchrus tribuloides L. In Belle Isle, June 24, 1905, No. 1919. Rare. The note under this name in the Michigan Flora refers entirely to *Cenchrus Carolinianus* Walt, which is very common.

SAVASTANA ODORATA var. FRAGRANS (Willd.) n. comb. (*Holcus fragrans* Willd. Sp. Pl. IV. 936, 1805). The plant in Michigan apparently is not exactly identical with the species; the above name should be adopted. Keweenaw Co., Aug. 21, 1890, No. 801; Rochester, May 23, 1910, No. 801a; Parkedale, May 19, 1912, No. 2555.

Anthoxanthum odoratum L. has been collected near Palmer Park by Mr. C. Billington.

Oryzopsis pungens (Torr.) Hitch. should replace the name *O. juncea* (Mx.) B. S. P. Bluffs in Keweenaw Co., May 30, 1884, No. 214.

O. racemosa (J. E. Sm.) Ricker should replace the name *O. melanocarpa* Muhl. Rochester, May 12, 1909, No. 2073.

Alopecurus aristulatus Mx. should replace A. *geniculatus* L. Belle Isle, June 4, 1895, No. 972; Detroit, June 17, 1911, No. 214a; Port Huron, June 23, 1918, No. 4969.

Sporobolus brevifolius (Nutt) Nash. should be transferred to Muhlenbergia, where it becomes *M. cuspidata* (Torr.) Rydb. Keweenaw Co., June 27, 1895, No. 848; and *Muhlenbergia squarrosa* (Trin.) Rydb. Keweenaw Co., July 1, 1895, No. 849½.

Sporobolus serotinus (Torr.) Gray should be replaced by *S. uniflorus* (Muhl) Scribn. & Merr. Keweenaw Co., Aug. 15, 1887, No. 526, and Aug. 20, 1887, No. 526a.

Agrostis stolonifera L. (*A. vulgaris* With). In the Species Plantarum p. 62, 1753, Linnaeus, under this name, combined the

two species later known as *A. vulgaris* With. and *A. verticillata* Vill. Two years later in 1755 in the Flora Suecica, page 22, he used the same name and description for a plant that was common in Sweden, thus himself fixing the type of his *A. stolonifera*. As *A. verticillata* was not known to occur in Sweden at that time, the name must perforce be construed as applying to the *A. vulgaris* which was common there. The panicle is slightly purplish and minutely roughish. Keweenaw Co., July 5, 1895, No. 856; Belle Isle, July 21, 1895, No. 856a.

A. STOLONIFERA forma HISPIDA (Willd.) n. comb. (*A. hispida* Willd. Sp. Pl. 1,370, 1797). Panicle long hispid. Keweenaw Co., July 5, 1895, No. 856 1/3.

A. STOLONIFERA var. PALUSTRIS (Huds.) n. comb. (*A. polymorpha* var. *palustris* Huds. Fl. Ang. 32, 1798; *A. alba* Lin. Sp. Pl. 63, 1753). Plants low, panicles short and pale (not violet). Keweenaw Co., July 5, 1895, No. 856½; Ypsilanti, June 30, 1892, No. 560a and No. 1,251½; Detroit, July 23, 1902, No. 1780; Parkedale, July 23, 1912, No. 2853; Belle Isle, Aug. 29, 1903, No. 1811.

A. STOLONIFERA var. PALUSTRIS forma DECUMBENS (Gaud.) n. comb. (*A. diffusa* Host. Gram. Austr. IV. t. 55. 1809; *A. alba* var. *decumbens* Gaud. Fl. Hel. 1. 188, 1828). Panicle violet colored. Keweenaw Co., Aug. 26, 1887, No. 560; Algonac, July 7, 1812, No. 2829. *A. stolonifera* var. *minor* (Vasey) Farwell belongs here. The plant was determined by Dr. Vasey as his *A. alba* var. *minor*. It is low with a very narrowly oblong panicle. Keweenaw Co., Aug. 29, 1887, No. 571.

A. STOLONIFERA var. MAJOR (Gaud.) n. comb. (*A. alba* var. *major* Gaud. Fl. Helv. 1, 189, 1828; *A. gigantea* Gaud. Agrost. I, 81, 1811.) A tall form with elongated panicles and broader leaves. Belle Isle, July 2, 1901, No. 1719.

A. stolonifera var. *coarctata* (Ehrh.) Pers. (*A. stolonifera* var. *maritima* (Lam.) Koch; *A. alba* var. *coarctata* (Ehrh.) Blytt.) Plant gray-green, stems prostrate and rooting at the joints, flowering branches erect, tufted or cespitose, panicle pale, contracted. Detroit, June 27, 1893, No. 560 b; Belle Isle, July 22, 1893, No. 560 c; Algonac, July 7, 1912, No. 2828. In the last edition of Gray's Manual the varietal name "maritima" is used for this plant, but the use of "coarctata" as a varietal name dates from Persoon, 1805, and antedates that of the other by 31 years.

A. stolonifera var. *stolonifera* (Sm.) Koch. (*A. stolonifera* var. *prorepens* Koch; *A. alba* var. *prorepens* Asch.; *A. alba* var. *stolonifera* G. F. W. Mey.) Similar to the last preceding but plant grass-green, more widely creeping and less tufted, flowering branches reclining or weakly ascending, not erect. Rochester, July 4, 1901, No. 560 d; Parkedale, July 4, 1918, No. 5032. The var. *coarctata* generally grows in rather dry places, while the var. *stolonifera* is usually found on wet grounds.

The above interpretation should replace *A. alba* and its var. *stolonifera* and *A. coarctata* of the Michigan Flora.

Calamagrostis confinis of the Michigan Flora becomes *C. inexpansa* A. Gr.

C. hyperborea Lange should be looked for along the south shore of Lake Superior, as it is known to occur in Minnesota and Northern Vermont.

C. Pickeringii var. *lacustris* (Kearney) Hitch. ranges along the Great Lakes and probably occurs in Michigan.

Holcus Authors not L. becomes Ginania Bub. *G. lanatus* (L.) Hubb. Velvet Grass. Detroit, August, 1908, No. 2062.

Eatonia Authors not Raf. becomes Reboulea Kunth. *R. obtusata* var. *lobata* (Lin.) O. A. F. doubtless occurs in Michigan as according to Gray's Manual it is the commonest form of the species in the north.

Triesetum subspicatum becomes *T. spicatum* (L.) Richter. Plants answering to the European type were collected at Lake Linden, Keweenaw Peninsula, in August, 1904, No. 1825½. A very rare species here, the common form being the var. *molle* (Mx.) Piper[1]; frequent along the rocky shores of the Peninsula, No. 658, August 16, 1888.

Avena sativa L. An escape from cultivation is to be found throughout the State, as are all the cultivated grains. They are all found with sufficient frequency to be listed as adventitious. Keweenaw county, August 15, 1888, No. 528; Ypsilanti, June 30, 1892, No. 528a; Belle Isle, August 26, 1892, No. 528b.

Spartina cynosuroides of the Michigan Flora becomes S. Michauxiana Hitchc. The common form of this species in Michigan is a plant with a panicle about a foot long, with numerous short

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[1]A larger form of the species with glabrous stems and leaves, but with the lowest sheaths rough, with short reflexed pubescens, is the var. majus (Vasey) n. comb. (*Trisetum majus* (Vasey) Rydb.) Sunset Peak, Colorado, August 30, 1911, No. 2455½.

spikes, three inches or less in length on short peduncles ½-¾ of an inch long, lemmas 2 toothed. River Rouge, September 15, 1918, No. 5134, and September 30, 1915, No. 4119; Belle Isle, October 19, 1894, No. 1487; Rochester, September 27, 1917, No. 4653. A long-spiked form may be known as *Spartina Michauxiana* Hitchc. var. SUTTIEI n. var. An extreme form with panicle over a foot and a half in length, with fewer, longer spikes 5-7 inches long on peduncles 1½-2 inches. Island Lake, July 16, 1905, No. 1487a. Also Dr. George Suttie, Orchard Lake, July 29, 1892, and Waterford, August 27, 1893. A slender form may be known as *Spartina Michauxiana* Hitchc. var. TENUIOR n. var. More slender than the species, 3-4 feet high, panicle 1-1½ feet or more long, spikes 1-3 inches in length, scattered, alternate, only slightly exceeding the internode, the uppermost usually shorter than the internode, the terminal often elongated (4-5 inches), sessile or on short (not exceeding ⅜ of an inch) peduncles; 1st glume, with an awn equal to its own length or shorter, ⅓-⅔ the length of the floret; palet about ½ line longer than its lemma and about equal to the second glume, which has an awn equal to its own length or shorter. Spikelet, exclusive of awn, about 4 lines long. In dry sandy fields at River Rouge, September 15, 1918, No. 5138. Apparently a transition towards *S. alterniflora*, which it strongly resembles, but the first and second glumes are prominently awned, and the keel of the lemma ends abruptly at the sinus of the two-toothed apex, which characters will place it here. The keels and awns are sparsely hispid, otherwise the floret is glabrous.

Atheropogon Muhl. is scarcely distinct generically from Bouteloua Lag. The species is *Bouteloua curtipendula* (Mx.) Torr. Dry hillsides near Rochester, July 30, 1914, No. 3807.

Phragmites Trin. is equivalent to Arundo L. The generic description of Linnaeus in the 5th ed. of the Genera, page 35, 1754, is of this genus. The species is *Arundo Phragmites*, L. Keweenaw county, August 8, 1888, No. 641. Parkedale, August 4, 1912, No. 3013. St. Clair Flats, September 27, 1909, No. 641a. Trichoon Roth, 1798, is also a synonym and has 14 years priority over Phragmites Trin. 1812, and is the proper name to be taken up for the genus by those who think Arundo L. equivalent to Donax Beauv. The name would be *Trichoon Phragmites* (L.) Schinz & Thell.

Triscuspis seslerionides becomes *T. flava* (L.) Hubbard.

Briza media L. Grosse Pointe, July 21, 1906, No. 1986.

Briza minor L. Grosse Pointe, July 21, 1906, No. 1987.

Poa flava (L.) of the Michigan Flora is now known as *P. palustris* L.

Panicularia Americana becomes *P. grandis* (S. Wats.) Nash.

P. fluitans (L.) O. K. is an European species, the typical form of which is found in only a few places in North America. Banks of Detroit River, October 2, 1900, No. 1697.

P. FLUITANS var. SEPTENTRIONALIS (Hichk.) n. comb. (*P. septentrionalis* Hitchk. Rhodora VIII, 211, 1906.) Has shorter florets and spikelets than the species. Detroit, July 7, 1907, No. 2033; Rochester, August 15, 1909, No. 2033a; Parkedale, August 4, 1912, No. 3044; Port Huron, June 23, No. 4961.

P. FLUITANS var. SEPTENTRIONALIS f. GLAUCA, *n. f.* Whole plant densely glaucous, Rockwood, June 16, 1918, No. 1893.

P. fluitans var. *angustata Vasey.* (*P. borealis* Nash.) Spikelets and florets still shorter, especially the former. Detroit, June 17, 1911, No. 2215½; Parkedale, July 19, 1914, No. 3727, and July 4, 1918, No. 3030; Bloomfield, June 29, 1918, No. 4999 (Farwell & Billington). These various forms show a perfect graduation from one extreme to the other and should therefore be treated as one species, rather than as two or more distinct species.

Puccinellia Parl. becomes Atropis Trin. The latter name has three years priority over Puccinellia, and is the name in general use by European botanists for this group of species. Our species becomes *A. airoides* (Nutt.) Holm.

Festuca ovina L. var. *duriuscula* (L.) Koch. f. *villosa* (Schrad.) As. & Gr. A form in which the lemmas are pubescent. Rochester, July 4, 1901, No. 1722; Algonac, May 24, 1914, No. 3641.

F. rubra heterophylla Hack. is now generally known as *F. occidentalis* Hook.

Bromus aspera Murr. Stevens, July 14, 1918, No. 5087.

Bromus brizaeformis Fisch. & Mey. Detroit, June 30, 1907, No. 2027.

Bromus hordeaceus var. *leptostachys* (Pers.) Beck. Parkedale, August 4, 1912, No. 3033a.

Bromus Japonicus Thunb. Ypsilanti, June 25, 1910, No. 2158½.

Bromus purgans L. This species is now generally considered to be distinct from *B. ciliatus* L. Keweenaw county, August 26, 1887, No. 563; Rochester, July 4, 1896, No. 563a; Detroit, August 18, 1907, No. 563b; Parkedale, July 14, 1912, No. 2856.

Bromus purgans var. *latiglumis* Shear (*B. altissimus* Pursh). Detroit, June 30, 1907, No. 2026; Franklin, September 23, 1918, No. 5161.

Bormus purgans var. *incanus* Shear (*B. incanus* (Shear) A. S. H.) Detroit, June 30, 1907, No. 2029; Rochester, October 10, 1918, No. 5194.

Bromus racemosus var. *commutatus* (Schrad.) Hook. f. (*B. commutatus* Schrad.) Ypsilanti, June 21, 1892, No. 1246; Belle Isle, July 21, 1892, No. 1246a; Parkedale, July 14, 1912, No. 2841½.

Bromus squarrosus L. var. *villosus* (Gmel.) Koch. Belle Isle, September 20, 1896, No. 1558.

Lolium L. In addition to the species listed in the Michigan Flora we have the following:

Lolium perenne var. *tenue* L. Detroit, July 22, 1911, No. 2219.

Lolium multiflorum Lam. Detroit, August 8, 1970, No. 2181.

Lolium festucaceum Link. Detroit, June 15, 1910, No. 2157.

The genus Agropyron is widely distributed in Michigan and is represented by many species and varieties. The revised list follows:

A. caninum (L.) R. & S. (*A. caninoides* (Ramalay) Beal.) Keweenaw county, June 27, 1895, No. 533b; Marquette, July 6, 1895, No. 533e; Detroit, September 8, 1897, No. 533f; Island Lake, July 16, 1905, No. 533g; Parkedale, July 4, 1918, No. 5029. A plant with long, slender, cylindrical spikes, spikelets appressed and florets long awned.

A. caninum var. *pubescens* Scrib & Sm. Keweenaw county, August 15, 1887, No. 533, and June 27, 1895, No. 533d; Rochester, July 24, 1910, No. 533h.

A. caninum var. *unilaterale* (Cassidy) Vasey. (*A. Richardsoni* (Trin.) Schrad.) A form with larger spikes, awns purplish and spikelets secund, or one-sided on the spike. Keweenaw county, June 27, 1895, No. 533a.

Agropyron divergens Nees (*Agropyron spictatum* (Pursh) Rydb. Fl. Mont. 61, 1900, not Scribn. & Sm. 1897). Keweenaw county, July 4, 1895, No. 851b.

Agropyron divergens var. *inerme* Scribn. & Sm. Keweenaw county, July 4, 1895, No. 851c.

A. biflorum (Brign.) *R. & S.* (A. *violaceum* (Hornem.) Lange). Keweenaw county, June 27, 1895, No. 533c.

A. tenerum Vasey. Keweenaw county, August 15, 1887, No. 532a and July 8, 1890, No. 760.

A. TENERUM var. NOVAE-ANGLIAE (Scribn. & Sm.) n. comb. (*A. repens* var. *Novae-Angliae* Scribn. & Sm. U. S. Bul. Agrost IV,—, 1897; *A. pseudorepens Scribn. & Sm.* l. c., 34; *A. Novae-Angliae Scribn.* in Fl. Vt.,—. 1900). Keweenaw county, July 3, 1895, No. 851a; Marquette, July 6, 1895, No. 851d.

Agropyron repens (Lin.) Beauv. This species is common throughout Michigan in many forms. Keweenaw county, August 15, 1887, No. 532; Ypsilanti, June 21, 1892, No. 532b; Belle Isle, July 23, 1892, No. 532c; Marquette, July 6, 1895, No. 532d; Parkedale, July 28, 1912, No. 2923, August 4, 1912, No. 2964.

Agropyron repens f. *geniculatum*, Farwell. Detroit, June 24, 1899, No. 1634.

Agropyron repens f. *stoloniferum*, Farwell. Detroit, June 24, 1899, No. 1635.

Agropyron repens var. *agreste*, Anders. Detroit, June 24, 1899, No. 1632.

Agropyron repens var. *nemorale*, Anders. Keweenaw county, July 18, 1890, No. 759; Detroit, June 24, 1899, No. 759a; Lake Linden, August 24, 1912, No. 3078; Rochester, June 23, 1912, No. 2927, and June 15, 1913, No. 3492½; Orion, July 7, 1918, No. 5049.

Agropyron repens var. *pilosum* Scribn. Detroit, June 24, 1899, No. 1633; Belle Isle, July 20, 1899, No. 1633a; Parkedale, July 28, 1912, No. 2950, and July 4, 1918, No. 5024.

Agropyron repens var. *littoreum* Anders. Keweenaw county, September 10, 1887, No. 595.

Agropyron dasystachyum (Hook.) Vasey. Keweenaw Peninsula, August 18, 1890, No. 794.

Agropyron dasystachyum var. *subvillosum* Scribn. & Sm. Keweenaw county, June 27, 1895, No. 794a.

Agropyron spicatum Scribn. & Sm. (*A. Smithii* Rydb. Fl. Mont., 64, 1900; *A. occidentale* Scribn. U. S. Agrost. Circ. XXVII 9, 1900; *A. repens* var. *glaucum* Scribn. Mem. Tor. Club. V. 57, 1895). The fact that Scribner & Smith misidentified the *Festuca spicata* of Pursh does not invalidate the above binomial for the species they described, since the binomial always applies to the species described. Had those authors based their new combination solely on synonymy (without a description) the name would, perforce, have been a synonym of Pursh's species, the one on which it would have been based. As I understand this species, it is characterized by its glaucous, bluish-green appearance, which makes it stand out boldly and conspicuously from all surrounding herbage. Rochester, June 23, 1912, No. 2828; Detroit, June 17, 1911, No. 2214b.

Agropyron spicatum var. viride, n. var. Larger and coarser than the species with a longer spike and larger spikelets, whole plant pale green, not at all bluish nor glaucous. Detroit, Mich., June 24, 1899, No. 851e; Keweenaw county, July 3, 1895, No. 851.

Triticum aestivum L., the Bearded Wheat, is adventive in southern Michigan. Detroit, June 17, 1895, No. 842a; Keweenaw county, August, 1894, No. 842.

Triticum aestivum var. muticum (Alef.) n. comb. (*Triticum vulgare* var. *muticum* Alef. Landw. Fl. 328, 1866). The Beardless Wheat is adventive in southern Michigan. Detroit, June 13, 1895, No. 841a; Keweenaw county, August, 1894, No. 841.

Triticum aestivum var. leucospermum (Körn.) n. comb. (*T. vulgare*. var. *leucospermum* Körn. *System.* Ubers, 10, 1873). Spikes densely velvety; the lemmas are awnless or short awned and velvety pubescent. Roadsides, near Rochester, June 30, 1912, No. 2799.

Hordeum vulgare L. Keweenaw county, August, 1894, No. 843; Detroit, July 8, 1905, No. 843a; Houghton, August 28, 1912, No. 3098; Parkedale, August 13, 1909, No. 843b.

Hordeum vulgare var. *distichon* (L.) Alef. Parkedale, August 13, 1909, No. 2100a.

Hordeum vulgare var. *hexastichon* (L.) Alef. Parkedale, August 13, 1909, No. 2100b.

Secale Cereale L. Sandy hillsides at Houghton, August 28, 1912, No. 3099.

Secale Cereale var. *vulgare* Körn. Belle Isle, June 20, 1893, No. 1373.

Secale Cereale var. *multicaule*. Körn. Rochester, June 8, 1909, No. 1373a.

Elymus Canadensis L. (*E. Canadensis* var. *glaucifolius* (Willd.) Torr.) Rather scarce. This glaucous plant is the Linnaean species. Detroit, September 17, 1896, No. 1556; Rochester, September 6, 1909, No. 1556a.

Elymus Canadensis L. var. PHILADELPHICUS (L.?) n. var. (*E. Philadelphicus* L. Cent. 1, No. 14, 1755? *E. Canadensis* Amer. authors not Lin.) The green, non-glaucous plant. Marquette, August 30, 1898, No. 1619½; Rochester, September 6, 1909, No. 1619b; Monroe Piers, August 10, 1909, No. 2184½.

ELYMUS CANADENSIS var. BRACHYSTACHYS (Scrib. & Ball.) n. comb. (*E. brachystachys* Scribn. & Ball. U. S. Agrost. Bull, XXIV, 47, fig. 21, 1901). Very common throughout southern Michigan. Belle Isle, August 11, 1893, No. 1409; Orion, August 29, 1895, No. 1409a; Island Lake, July 16, 1905, No. 1929½; Rochester, August 15, 1909, No. 2109½.

ELYMUS CANADENSIS var. ROBUSTUS (Scribn. & Sm.) n. comb. (*E. robustus* Scribn. & S. M. U. S. Agrost. Bull, IV, 37, 1897). Has been reported from various places. The type of the species according to the Linnaean description and explanatory remarks can be no other than Willdenow's *E. glaucifolius*. The non-glaucous plant that has been passing as *E. Canadensis* L., may be *E. Phildelphicus* L. (?) and may be considered as a good variety of the former. *E. robustus* differs from the var. *Philadelphicus* only in a denser spike, less alternate at base, which is scarcely a specific character. *E. brachystachys* differs in having the glumes and lemmas hispidulous or glabrate, characters that are only of varietal rank.

Elymus striatus Willd. var. *villosus* (Muhl.) A. Gr. This variety should be maintained as valid for the same reason that var. *brachystachys* is maintained. In the species the spikelets are hispid and in the variety they are hirsute. On this slender character *E. brachystachys* (Scribn. & Ball) is maintained as a valid species by some, but in the case of *E. villosus Muhl*, an exactly parallel case, the name is reduced to the limbo of synonymy. The species is reported as occurring throughout Michigan. The only

form I have seen is the variety. Belle Isle, October 19, 1894, No. 1490.

Asperella Humb. has three years priority over Hystrix Moench.; the species is *Asperella Hystrix* (L.) Humb. Ypsilanti, July 23, 1891, No. 1174; Belle Isle, July 21, 1892, No. 1174a.

CYPERACEAE.

Cyperus inflexus Muhl. should be *C. aristatus* Rottb.

Cyperus esculentus var. *leptostachyus* Boeckl. is characterized by its much elongated spikelets (¾-1½ in.). Near Birmingham, October 13, 1918, No. 5214.

Cyperus filiculmus Vahl is common on dry sterile grounds. Parkedale, August 23, 1914, No. 3845. The var. *macilentus* Fernald is found in same situations, but is much less common. Near Ypsilanti, July 31, 1891, No. 1189; Detroit, July 19, 1894, No. 1189a; Rochester, July 4, 1896, No. 1189b, and September 28, 1911, No. 2956; Marl Lake, August 15, 1917, No. 4546.

Trichophyllum Ehrh. has 21 years priority over Eleocharis R. Br. Presumably the reason Ehrhart's name has been neglected may be found in the generally conceded preponderating weight of the influence inseparable from the authority that goes with the name of Robert Brown, aided, no doubt, by the ex-post facto laws passed by botanical congresses, the members of which have had no inclination to learn the many new names for old species that it would be necessary to adopt in case the changes were made, always a sign of retrogression, rather than of progression. The names of Michigan species not already transferred follow:

T. INTERSTINCTUM (Vahl.) n. comb. (*Scirpus interstinctus* Vahl. Enum. II, 251, 1806).

T. MUTATUM (L.) n. comb. (*Scirpus mutatus* L. Amoen. Acad. V. 391, 1760).

T. ROBBINSII (Oakes) n. comb. (*Eleocharis Robbinsii* Oakes, Hovey's Mag. VII, 178, 1841).

T. OLIVACEUM (Torr.) n. comb. (*Eleocharis olivacea* Torr. Ann. Lyc. N. Y. III 300, 1836).

T. OVATUM (Roth) n. comb. (*Scirpus ovatus* Roth. Catal. Bot. I 5, 1797). On the Keweenaw Peninsula, but scarce. August 22, 1887, No. 547.

T. OBTUSUM (Willd.) n. comb. (*Scirpus obtusus* Willd.

Enum. 76, 1809). Very common throughout. Keweenaw county, August 22, 1887, No. 547a; Belle Isle, August 6, 1882, No. 547b; Royal Oak (Zoo Park), July 13, 1916, No. 4320.

T. PALUSTRE var. GLAUCESCENS (Willd.) n. comb. (*Scirpus glaucescens* Willd. Enum. 76, 1809). Keweenaw county, July 25, 1890, No. 772; Ypsilanti, June 21, 1892, No. 772a; Detroit, July 10, 1892, No. 772b, and July 7, 1893, No. 772c; Tacoma, July 2, 1916, No. 4270. A slender, filiform condition of the species.

T. PALUSTRE var. CALVUM (Torr.) n. comb. (*Eleocharis calva* Torr. Fl. N. Y. II, 346, 1843). A low slender form without bristles. Grosse Pointe and Belle Isle, July 21, 1906, No. 1988.

T. PALUSTRE var. VIGENS (Bailey) n. comb. (*Eleocharis palustris* var. *vigens* Bailey, Journ. N. Y. Micros. Soc. V 104, 1889). A very stout and rigid form of the species. Keweenaw county, July 25, 1890, No. 773.

T. ENGELMANNI (Stued.) n. comb. (*Eleocharis Engelmanni* Stued. Syn. Pl. Cyp. 79, 1855). Detroit, October 2, 1900, No. 1696.

T. ACICULARE (L.) n. comb. (*Scirpus acicularis* L. Sp. Pl. 48, 1753). Keweenaw county, September 6, 1888, No. 676; Belle Isle, August 6, 1892, No. 676a; Orion, August 29, 1895, No. 676b; Ypsilanti, July 31, 1891, No. 676c; Lake Linden, August 21, 1812, No. 3081; Tacoma, July 2, 1916, No. 4268.

T. TENUIS (Willd.) n. comb. (*Scirpus tenuis* Willd. Enum. 76, 1809). Keweenaw county, August 18, 1890, No. 548a.

T. ACUMINATUM (Muhl.) n. comb. (*Scirpus acuminatus* Muhl. Gram. 27, 1817). Keweenaw county, August 22, 1887, No. 548.

T. INTERMEDIUM (Muhl.) n. comb. (*Scirpus intermedius* Muhl. Gram. 31, 1817).

IRIA CASTANEA (Mx.) n. comb. (*Scirpus castaneus* Mx. Fl. Bor. Amer. I, 31, 1803).

IRIA AUTUMNALIS (L.) O. K. var. GEMINATA (Lestib. & Nees) n. comb. (*Trichelostylis geminata* Lestib. & Nees, Fl. Bras. II pt. 1, pp. 79 and 80, 1842). Iria Richard has a year's priority over Fimbristylis Vahl; Otto Kunze took up the name, but changed the spelling to Iriha. The old *F. autumnalis* (L.) R. and S. has been split and that part of the species retaining the old name is of southern range. That part of the species extending further north

has been known as *F. geminata* (Lestib. & Nees) Kunth or *F. Frankii* Steud. The characters used to distinguish the two forms are so slight that it seems better to retain them as variations of the same species. The Michigan plant, presumably, is of the varietety geminata, as its range is too far north for it to be likely that it should belong to the typical form of the species. I haven't seen it.

Scirpus lacustris L. has been restricted to the Old World. The American plant is mostly the *S. validus* Vahl. Keweenaw county, September 6, 1888, No. 677; Ypsilanti, June 23, 1891, No. 677a; Detroit, August 6, 1892, No. 677b, and July 18, 1912, No. 2874; Parkedale, August 4, 1912, No. 2994; Grosse Isle, August 20, 1916, No. 4387; Tacoma, September 23, 1917, No. 4624.

S. occidentalis (Watson) Chase. Keweenaw county, August, 1904, No. 1834; Parkedale, July 28, 1912, No. 2918; Marl Lake, August 13, 1916, No. 4377.

S. occidentalis var. *congestus* O. A. F. Marl Lake, July 9, 1916, No. 4294, and August 13, 1916, No. 4376.

S. heterochaetus Chase. Another segregate of the old *S. lacustris* L., which is said to occur in Michigan.

S. atrovirens Muhl var. *pycnocephalus* Fernald. Parkedale, August 4, 1912, No. 3021. The infloresence in this has been reduced to a glomerate head.

S. rubrotinctus Fernald. This is the old *S. microcarpus* Presl. Keweenaw county, September 12, 1887, No. 549a.

S. sylvaticus L. Keweenaw county, August, 1904, No. 1824; Junior, July 13, 1918, No. 5079.

S. Georgianus Harper. Accredited to Michigan in Gray's Manual.

S. Cyperinus (L.) Kunth. *S. lineatus* Mx. and allied species should be removed to Eriophorum. The elongated bristles exclude them from Scirpus, but if they are to be retained in that genus, then all distinctions between it and Eriophorum break down and both should be united. It would be better to restore the old genus Trichophorum for this group of plants than to retain them in Scirpus.

E. lineatum (Mx.) Bth. & Hk. f. Keweenaw county, August 21, 1890, No. 799; Ypsilanti, June 23, 1891, No. 799a; Detroit, July 21, 1892, No. 799b.

E. Cyperinum L. Keweenaw county, September 6, 1888, No. 678; Detroit, September 15, 1899, No. 678a.

E. Cyperinum var. *pelium* (Fernald) O. A. F. Keweenaw county, August 8, 1890, No. 756b, and August 15, 1901, No. 1736; Detroit, August 4, 1896, No. 756c; Marl Lake, August 13, 1916, No. 4358.

E. Cyperium var. pelium *f.* condensatum (Fern.) n. comb. (*Scirpus cyperinus* var. *condensatus* Fernald; Gray Manual, 195, 1908). Accredited in the Manual to Michigan.

E. Cyperinum var. *laxum* Wats. & Coult. (*Scirpus Eriophorum* Mx. Fl. Bor. Am. I, 33, 1803). Detroit, September 18, 1902, No. 1795.

E. Cyperinum var. *pedicellatum* (Fernald) O. A. F. (*E. cyperinum* var. *laxum* Wats & Coult. in part). Keweenaw county, August, 1904, No. 1826½.

E. Cyperinum var. pedicellatum f. grande n. comb. (*Scirpus atrocinctus* var. *grandis* Fernald, and *S. pedicellatus* var. *pullus* Fernald. Gray Manual, 195, 1908). Keweenaw county, August, 1902, No. 1788.

E. Cyperinum var. *atrocintum* (Fernald) O. A. F. (*E. Cyperinum* var. *laxum* Wats. & Coult. in part). Keweenaw county, July 18, 1890, No. 756.

E. Cyperinum var. atrocinatum f. brachypodum (Fernald) n. comb. (*S. atrocinctus* var. *brachypodus* Fernald, Proc. Am. Acad. XXIV, 502, 1899). Keweenaw county, August 18, 1890, No. 756a.

Typical *E. vaginatum* L. is now restricted to the Arctic regions. The Michigan material is referred to two varieties. *E. vaginatum* var. *humile*, Turcz. (*E. callitrix* Cham.) Keweenaw county, August 22, 1887, No. 55, and *E. vaginatum* var. *opacum* Bjornstr. Lakeville, Farwell and Billington, June 2, 1918, No. 4907.

E. gracile Koch. Keweenaw county, August 22, 1887, No. 551.

E. gracile var. *paucinervium* Engelm. Keweenaw county, August, 1901, No. 1735. Neither form of the species is common. The variety has the blade of the uppermost leaf longer than its sheath, while in the species it is shorter.

E. polystachion L. (*E. angustifolium* Roth). Keweenaw county, September 10, 1888, No. 691.

E. polystachion var. *majus* (Schultz) As. & Greb. This variety is accredited to Michigan in Gray's Manual.

E. POLYSTACHION var. VIRIDI-CARINATUM (Engelm) n. comb (*E. latifolium* var. *viridi-carinatum* Engelm. Am. Journ. Sci XLVI, 103, 1844). Keweenaw county, July 12, 1890, No. 743; Parkedale, June 2, 1912, No. 2614.

Phaeocephalum Ehrh. (Triodon Pers. and Rynchospora Vahl) appears to be the oldest name for the genus. The Michigan species are the following:

P. ALBUM (L.) n. comb. (*Schoenus albus* L. Sp. Pl. 44, 1753). Keweenaw county, August 22, 1887, No. 552; Rochester, August, 1908, No. 552a; Marl Lake, August 13, 1916, No. 4374.

P. ALBUM var. MARCUM (Clarke) n. comb. (*Rynchospora alba* var. *macra* Clarke in Britt. Trans. N. Y. Acad. Sci. XI, 88, 1892).

P. CAPILLACEUM (Torr.) n. comb. (*R. capillacea* Torry Comp. 41, 1826). Orion, August 29, 1895, No. 884; Marl Lake, August 13, 1916, No. 4366; Parkedale, July 14, 1912, No. 2858.

P. CAPILLACEUM var. *levisetum* (E. J. Hill) n. comb. (*R. capillacea* var. *leviseta* E. J. Hill; Gray's Manual 201, 1908). Orion, August 29, 1895, No. 911.

P. GLOMERATUM (L.) (*Schoenus glomeratus* L. Sp. Pl. 44, 1753) var. MINUS (Britt.) n. comb. (*R. glomerata* var. *minor* Britt. Trans. N. Y. Acad. Sci. XI 89, 1892). The variety only is found in Michigan. Ypsilanti, August 12, 1891, No. 1199; Detroit, July 27, 1893, No. 1199a; Keweenaw county, August, 1904, No. 1199b; Parkedale, July 28, 1912, No. 2919.

P. CYMOSUM (Ell.) n. comb. (*R. cymosa* Ell. Sketch. I 58, 1816).

P. FUSCUM (L.) n. comb. (*Schoenus fuscus* L. Sp. Pl. 1664, 1763).

Carex sterilis Willd. (*C. scirpoides* Schk.) There are two forms of this species found here in Michigan. One is found in open bogs and swamps and along the borders of woods, and the other in dense boggy woods such as tamarack swamp, cedar swamps, etc., where sphagnum is liable to be found. The former has the stiff, wiry habit of *C. echinata*, but the leaves are narrower, 1 to 1¼ lines wide and the perigynia are broader, the width being more than ½ the length, and gradually tapering into the

inconspicuously bifid beak, the edges being strongly roughened, about 1 1/4 lines long by about 7/8 line wide, finally becoming yellowish or brownish. Often dioecious. This form probably is typical of Willdenow's *C. sterilis*. The latter is slender, lax, with leaves only 1/2 as wide, the green perigynia abruptly contracted into the beak, the edges being minutely roughened; it is essentially the plant described by Bailey as *C. interior*. It seems to the present writer that the difference outlined is mainly that which might be sought in a shade form of an ordinarily sunlight plant, and therefore this form should be properly treated as a variation rather than as a distinct species. It may be known as *C. sterilis* Willd. f. FLEXIBILIS, n. f. Lakeville, June 2, 1918, No. 4887.

Carex vulpinoidea Mx. var. *ambigua* Barratt. A rare form of the species in Michigan. Spikes yellowish brown and forming a strong contrast to the usually green or brownish spikes of the species. Detroit, June 16, 1900, No. 1670; Junior, July 13, 1918, Farwell & Billington, No. 5074.

Carex stricta Lam. var. *strictior* (Dew.) A. Gr. (*C. stricta* var. *curtissima* Peck). Does not form dense tussocks as does the species. Keweenaw Point, August 26, 1887, No. 565, and August, 1904, No. 1831½; Ypsilanti, July 29, 1906, No. 565b; Orion, June 9, 1918, No. 4933.

Carex stricta Lam. var. *xerocarpa* (S. H. Wright) Britton. In moist woods near Rochester, May 28, 1918, No. 4875, and No. 4882c.

Carex aurea Nutt f. COLORATA n. f. The scales pale brown. In tamarack swamps at Lakeville, June 2, 1918, No. 4882g.

Carex Harperi Fernald (?). These plants appear to be intermediate between this species and *C. leptalea* Wahlenb. The persistent scales are brownish and mucronate instead of white and acuminate, but the perigynia are slender, and gradually tapering at the base and the achenes are puncticulate and sharply trigonous as in *C. Harperi*. Orion, June 9, 1918, No. 4929; Parkedale, July 4, 1918, No. 5034.

Carex umbellata Schkuhr. var. *brevirostris* Boot. Banks of the Huron River at Ypsilanti, May 19, No. 4831, Billington & Farwell.

Carex Pennsylvanica Lam. var. *lucorum* (Willd.) Fernald. Port Huron, June 23, 1918, No. 4985; Detroit, June 11, 1903, No. 1800.

Carex paupercula Mx. var. *irrigua* (Wahl.) Fernald. In bogs. Keweenaw county, September 12, 1887, No. 608, and August 26, 1912, No. 3090; Orion, July 7, 1918, No. 5055, Farwell & Billington.

Carex paupercula Mx. var. *pallens* Fernald. Keweenaw county, July 5, 1895, No. 854½; Orion, July 7, 1918, No. 5040a, Farwell & Billington.

Carex laxiflora Lam. var. *leptonervia* Fernald. This is accredited to Michigan in Gray's Manual. Port Huron, June 23, 1918, No. 4977, Farwell & Billington. Rare. In rich woods.

Carex flava L. var. *rectirostra* Gaudin (*C. flava* var. *graminis* Bailey). Borders of Marl Lake, rare, June 9, 1918, No. 4923; Keweenaw county, August 24, 1888, No. 672; Dead Lake, July 16, 1910, No. 672b.

Carex lanuginosa Mx. var. *Kansana* Britt. Plant with leaves ½ narrower than in the species, spikes longer, narrower, and tapering at base. Roadsides near Rochester, May 28, 1918, No. 4882f.

Carex retrorsa Schwein var. *Robinsonii* Fernald. A form with slender spikes. Rare. Ypsilanti, July 23, 1891; Dearborn, July 6, 1918, No. 5029.

Carex lupulina Muhl var. *polystachya* Schw. & Torr. Common at Junior in low moist grounds. July 13, 1918, No. 5064, Billington & Farwell.

Carex vesicaria L. Roadside ditches near Lakeville. The typical species not before reported from Michigan insofar as I am aware. June 2, 1918, No. 4883, Farwell & Billington.

LILIACEAE.

Erythronium Americanum Ker. var. Bachii, n. var. A form in which the lower half of the perianth segments and stamens are purplish brown or magenta. Near Redford, May 19, 1918, No. 4851. Named for Mr. Bach of Detroit, who found it in considerable numbers.

Trillium recurvatum Beck. Perhaps the rarest Trillium in Michigan. Near Rochester, May 15, 1918, No. 4822. Has been reported from Ypsilanti.

TRILLIUM CERNUUM var. DECLINATUM (A. Gr.) n. comb. (*T. erectum* L. var. *declinatum* A. Gr. Manual, 523, 1868). This

plant certainly is closely related to *T. cernuum* L. In the "copper region" of Michigan, where both are plentiful, they seem to intergrade, and at times it is very difficult to determine to which form certain individuals should be referred. It seems better to regard them as extremes of one species, rather than as two. Keweenaw county, July 8, 1886, No. 411; Ypsilanti, May 19, 1918, No. 4848.

T. cernuum L. var. *declinatum* A. Gr. f. WALPOLEI n. f. Petals and often the filaments and stigmas deep purple. Named for Mr. B. A. Walpole of Ypsilanti, one of the discoverers. Ypsilanti, May 19, 1918, No. 4849.

T. cernuum L. var. *declinatum* A. Gr. f. BILLINGTONII n. f. Flowers brown with a slight tinge, here and there, of a purplish color. Named for one of the discoverers, Mr. Cecil Billington, of Detroit. Ypsilanti, May 19, 1918, No. 4850.

Trillium grandiflorum (Mx.) Salisb. f. ROSEUM n. f. Flowers rose colored. Ypsilanti, May 19, 1918, No. 4847; Birmingham, May 18, 1902, No. 767c. *T. grandiflorum* is common throughout eastern North America. I have seen acres of it and probably many thousands of individuals without a rose-colored flower to change the monotony of the pure white of the open woods as far as the eye could detect them. In some localities rose-colored flowers are more common than the normal white flowered form, but such places are few in comparison. In these localities, the rose-colored individuals appear as though they had been rose-colored from the beginning and do not lose the color even in the withered and shrunken petals. The near-by white flowered forms when faded and wrinkled up are not rose-colored but present the dirty-white or dull yellowish-white color characteristic of such conditions. It seems probable that the "rose" and the "white" in this species are as permanent as the "purple" and "white" in *T. erectum* L. or in *T. cernuum* L.

T. grandiflorum var. *obovatum* f. ALBIFLORUM n. f. (*T. grandiflorum* var. *obovatum* O. A. F. Mich. Acad. Sci. Rept. 1918, p. 157; *T. grandiflorum* var. *parvum* Gates Ann. Mo. Bo. Gard. IV, 58, 1917, as of the white flowered forms). Flowers white. Farmington, May 19, 1917, No. 4413. The remarks above concerning the color forms of the species apply equally well to the color forms of this variety. I may add that of the myriads of individuals observed, I have never seen the rose color beginning

to develop in a white petal after it had begun to fade. On the other hand, I have never examined a bud to ascertain if the petals at that stage of development were of a rose color.

CASTANEACEAE.

Quercus stellata Wang. The Post Oak reported in the 20th Annual Report, Mich. Acad. Sci., p. 172, as *Quercus lyrata* Walt, should have been listed as *Quercus stellata* Wang.

ARISTOLOCHIACEAE.

The flowers of the genus Asarum are said to be 3-merous. Mr. Walpole, of Ypsilanti, has found *A. Canadense* L. near that city to be 4-merous almost as frequently as it is 3-merous.

PERSICARIACEAE.

Polygonum amphibium L. (*P. amphibium* var. *aquaticum* Leyss. Fl. Hal. 391, 1761; *P. amphibium* var. *palustre* Weig. Fl. Pom. 255, 1769, according to Ascherson and Graebner; *P. amphibium* var. *natans* Moench. Enum. Pl. Hass. 28, 1777). A glabrous aquatic with narrowly oblong-lanceolate acute floating leaves, usually narrowed but occasionally rounded at base. Keweenaw county, September 4, 1885, No. 352.

P. amphibium f. *terrestra* (Leyss.) S. F. Blake. (*P. amphibium* var. *terrestre* Leyss. Fl. Hal. 391, 1761; *P. amphibium* var. *erectum* Kittel. Tauschenb. 303, 1853; *P. amphibium* var. *salicifolium* Schur, Enum. Pl. Transs. 583, 1866). A terrestrial, decumbent to erect, strigose pubescent variation. Algonac, September 13, 1915, No. 3895; Detroit, September 20, 1918, No. 5151.

P. amphibium var. *marginatum* n. var. Differs from the typical form of the species only in having a foliaceous border on the stipular sheaths. Keweenaw county, September 4, 1885, No. 351.

P. AMPHIBIUM var. MARGINATUM f. HARTWRIGHTII n. comb. (*P. amphibium* var. *Hartwrightii* (A. Gr.) Bissell, Rhodora IV 105, 1902). A terrestrial, decumbent to erect, strigose pubescent variation. Belle Isle, August 2, 1892, No. 351a; Lakeville, September 2, 1901, No. 351b; Parkedale, July 30, 1914, No. 3812; Bloomfield, June 29, 1918, No. 5002.

P. amphibium var. *natans* Mx. (*P. coccineum* Muhl. and var. *aquaticum* Willd. *P. amphibium* var. *coccineum* (Muhl.) O. A. F.

Aquatic; leaves ovatelanceolate, usually cordate; plant glabrous. Belle Isle, September 3, 1892, No. 352a.

P. amphibium var. *natans* f. EMERSUM (Mx.) n. f. (*P. amphibium* var. *emersum* Mx. Fl. Bor. Amer. I, 240, 1803; *P. coccineum* var. *terrestre* Willd. Enum. I, 429, 1809; *P. amphibium* var. *terrestre* Torr. F.l N. Y. II, 149, 1843; *P. amphibium* var. *Muhlenbergii* Meisn. in DC. Prodr. XIV, 116, 1856). An erect, terrestrial, strigose pubescent form. Belle Isle, October 19, 1893, No. 1452; Grosse Isle, August 11, 1909, No. 1452a. It has been said that the terrestrial forms are but mere phases and may be found on the same rhizomes which produce the aquatic plants. My experience shows that the terrestrial forms are rather common in Michigan, while the aquatics are infrequent. I have never seen the two phases associated or even in close proximity to each other.

Polygonum aviculare L. var. *vegetum* Ledeb. Along roadsides in the Bloomfield hills. Prostrate. Billington & Farwell, June 29, 1918, No. 1989. Also in Detroit, where the plants were ascending or erect.

BLITACEAE.

Chenopodium album L. A common and variable weed throughout the State. Ypsilanti, June 17, 1891, No. 1152; Detroit, July 16, 1892, No. 1152a; Keweenaw county, June 26, 1895, No. 1152b. The following varieties are also distinguishable:

C. album var. *opulifolium* (Schrad.) G. Meyer. (*C. viride* L.). Leaves thinner, less toothed and acute or entire and obtuse, greener, the lower as broad as long. Keweenaw county, August 12, 1885, No. 311; Ypsilanti, September 2, 1891, No. 311c; Belle Isle, July 21, 1892, No. 311d.

C. album var. *integerrimum* S. F. Gray (*C. lanceolatum* Muhl). Leaves usually narrowly lanceolate and entire. Detroit, September 9, 1895, No. 311b.

C. album var. *viridescens* St. Am. (*C. paganum* Reichenb). Leaves green and panicle lax. Oakwood, September 15, 1918, No. 5135.

Salsola Kali L. var. *Caroliniana* (Walt.) Nutt. Leaves short, plant glabrous. Rochester, October 6, 1918, No. 5187.

S. Kali var. *tenuifolia Tausch*. Leaves long and filiform, plant usually rough. Rochester, October 6, 1918, No. 5188.

RANUNCULACEAE.

Anemone cylindrica A. Gr. f. ALBIDA n. f. Sepals petal-like white, thin, obovate, oval, or ovate, 4-8 lines in length. Parkedale, July 4, 1918, No. 5016.

POMACEAE.

Crataegus pruinosa (Wendl.) C. Koch. Near Redford, May 24, 1918, No. 4863.

C. Jesupi Sarg. Franklin, Sept. 22, 1918, No. 5162.

ROSACEAE.

Rosa Carolina L. (*R. humilis* Marsh.) One of our common wild roses and immensely variable. The leaflets vary from obovate or oblanceolate through oval to elliptic and elliptic-lanceolate, obtuse or acute; it is no uncommon thing to see all forms on a single individual; the serratures of the leaflets are more or less glandular-tipped in all the forms and in one variety they are somewhat glandular ciliate; the petiole and rachis vary from pubescent, with or without glands to glabrous or glandular; the prickles are usually slender, but in one variety they are frequently stouter and recurved. All forms intergrade one into another and are best treated as a single species. Dearborn, July 6, 1918, No. 5021a; Orion, July 7, 1918, No. 5044a; Bloomfield, September 8, 1918, No. 5107.

R. Carolina var. GLANDULOSA (Crepin) n. comb. (*R. parviflora* var. *glandulosa Crepin*, Bull, Soc. Bot. Belg. XV, 68, 1876; *R. serrulata* Raf. Ann. Gen. Sci. Phys. V. 218, 1820.) *Rachis* usually glandular and some of the leaflets with some glandular ciliation. Parkedale, July 4, 1918, No. 5039; Orion, July 7, 1918, No. 5046a; Bloomfield, September 8, No. 5115.

R. CAROLINA var. GRANDIFLORA (Baker) n. comb. (*R. humilis* var. *grandiflora Baker;* Willm. Gen. Rosa I, 207, 1911, and *R. obovata* Raf. according to Rydberg in N. A. Flora XXII, 499, 1918.) Leaves usually obovate and obtuse with a cuneate base. Perhaps the most distinct of the various forms. Keweenaw county, September 10, 1888, No. 691; Parkedale, July 4, 1918, No. 5036.

R. CAROLINA var. LUCIDA (Ehrh.) n. comb. (*R. Virginiana* Miller, Gard. Dict. No. 10, 1768; *R. humilis* var. *lucida* (Ehrh.) Best, Bull. Tor. Club XIV, 256, 1887). In this variety the prickles

are usually stouter than in the others, and often reflexed or recurved. Parkedale, July 4, 1918, No. 5038; Orion, July 7, No. 5045a.

LEGUMINACEAE.

Lespedeza capitata Michx. var. *longifolia* (DC.) T. & G. Rochester, October 5, 1913, No. 3538; Bloomfield, September 8, 1918, No. 5108.

Lathyrus palustris L. var. *myrtifolius* (Muhl.) A. Gr. f. PALLIDA n. f. Flowers white or whitish. Open tamarack swamps. Orion, July 7, 1918, No. 5052.

SAPINDACEAE.

Aesculus glabra Willd. Bloomfield, Oakland county, Mr. C. Billington, 1917; May 25, 1918, No. 4865.

ZIZIPHACEAE.

Rhamnus cathartica L. At some time or other a hedge was planted on a farm in the Bloomfield Hills region. The hedge, or what is left of it, is still a score or so of yards in length, impenetrable, and about 20 feet in height. The region in the vicinity of the hedge is well covered with young plants, which goes to prove that it is spreading from seed and has become naturalized. September 8, 1918, No. 5109.

TILIATACEAE.

Tilia Americana L. The leaves of the typical species are usually described as glabrous or nearly so. I have seen no Lindens in southern Michigan with glabrous leaves; a leaf here and there is quite densely pubescent with stellate hairs. Generally, however, the leaves are covered on the under side with a fine pubescence with a trace of stellate and long simple hairs. There are two well defined and easily recognized forms based on size and shape of leaves. The large leaved form at flowering time has the leaves 4 to 5 inches wide and 5 or 6 inches long, measured along the midvein, ovate to ovate-oblong, obliquely, truncate or obliquently cordate, style, peduncle and usually the bract glabrous, pedicles stellate pubescent, but branches of the cyme glabrous. This probably is the *T. neglecta* Spach, Ann. Sci. Nat, II, 2, p. 140, t. 15, 1834, and may be known as *T. Americana* var. SCABRA n. var.

Junior, July 13, 1918, No. 5061. Widely distributed in southern Michigan. The small leaved form has leaves about ½ as large, about 1½ to 3 inches long and the same in width, round ovate and more generally obliquely cordate, style pubescent at base, peduncle glabrous but bract usually more or less stellate pubescent, pedicels stellate pubescent and cyme branches more or less so. This may be known as *T. Americana* var. SCABRA f. MICROPHYLLA n. f. Frequent at Rochester and vicinity, July 13, 1918, No. 5062, and Oct. 28, 1917, No. 4803. Large leaves on succors may be over a foot in diameter.

CORNICULATACEAE.

Epilobium oliganthum Mx. (*E. lineare* var. *oliganthum* (Mx.) Trelease). The typical, low, simple, few-flowered form with opposite, linear leaves I have not seen in Michigan. The common form here is the common form of the species with linear, strongly revolute leaves, frequently revolute clear to the midvein, densely bushy-branched above, forming a compact top especially during the fruiting season. This is the *E. lineare* Muhl. Cat. 39, 1813; *E. rosmarinifolium* Pursh Fl. I, 259, 1814; *E. leptophyllum* Raf. and *E. densum* Raf. Desv. Jour. Bot. II, p. 271, 1814; *E. squamatum* Nutt. Gen. I, 250, 1818; *E. palustre* var. *albescens* Richards. Frankl. Journ. 12, 1823; *E. palustre* var. *albiflorum Lehm.* in Hook Fl. Bor. Amer. I, 207, 1833; and *E. palustre* var. *lineare* A. Gr. Man., 130, 1856. It may be known as E. OLIGANTHUM var. ALBESCENS (Richards.) n. comb. In swamps near Bloomfield, Sept. 8, 1918, No. 5102. Another form, somewhat taller (about 2 feet high), much more slender and divaricately branched, forming a broad, open top, has lanceolate flat leaves (primary, 1½-2 lines wide), margin barely recurved, and may be known as *Epilobium oliganthum* var. GRACILE n. var. Swamps near Bloomfield, Sept. 8, 1918, No. 5103.

UMBELLATACEAE.

Thaspium trifoliatum (L.) A. Gr. In *Rhodora* for March, 1918, Mr. S. F. Blake, in a discussion of this species and the Clayton Herbarium, makes the statement that Nuttall's *Thaspium aureum* is ultimately based upon the *Smyrnium aureum* L. and consequently Nuttall's name must be considered as a synonym of *Zizia aurea* (L.) Koch. In making this statement Mr. Blake is making

Nuttall say what he never intended to say, what he never did say, and probably what he never thought of saying. The conclusion of Blake would be a legitimate one if Nuttall had proposed the species on synonyms alone, as, for instance, has been done in Vol. V of the Memoir of the Torrey Botanical Club for a large number of new combinations. But Nuttall gave a fairly accurate description of his species, so accurate, indeed, that it will tax the ingenuity of the most expertly critical of modern American botanists to discover any resemblance in the fruit of *T. aureum* as described by Nuttall to that of *Zizia aurea* (L.) Koch or to reconcile the reduction of the former to the latter. *Thaspium aureum* Nutt. is exemplified by Nuttall's description, not by the synonym of Pursh listed after the description. Nuttall wrote an entirely new and accurate description based upon the species he had before him, not upon Pursh's description. That he quoted Pursh's *Smyrnium aureum* as a synonym of his own species is due to a mistaken identification and in no wise can separate Nuttall's name from his species, which is a true Thaspium. Blake also claims that *Thapsia trifoliata* L. is the same as the purple flowered *Thaspium atropurpureum* Nutt. because the specimen in the Clayton herbarium is a purple flowered plant, the Linnaean species being based through the Gronovian reference on the Clayton specimen. The Gronovian description calls for a plant with crenate leaflets; those of *T. atropurpureum* Nutt. are serrate. The purple flowered species cannot be typical *Thapsia trifoliata* L. because it does not answer to the description. Of the yellow-flowered species generally known as *Thaspium aureum* Nutt. there are two forms, one with serrate leaflets typical of Nuttall's species and one with crenate leaflets which is typical *T. trifoliatum* (L.) A. Gr. This latter is occasional in southern Michigan. A form with simply ternate stem leaves and cordate radical leaves has often been reported by some as *Zizia cordata* (Walt.) D. C. Rockwood, June 16, 1918, No. 1952; Ypsilanti, May 19, 1918, No. 1827½. The Ypsilanti plant has the radical leaves ternate and lower stem leaves biternate.

Thaspium trifoliatum var. *aureum* (Nutt.) Britt. This is the yellow-flowered form with serrate leaflets. Ypsilanti, May 19, 1918, No. 4827; Rochester, May 28, 1918, No. 1882b. The *Thaspium atropurpureum* Nutt. Gen. 1, 196, 1918, is but a simple flowered form of *T. aureum* Nutt. and may be known as *T. tri-*

foliatum var. *aureum* f. *atropurpureum* (Desr.) n. f. I have not seen it from Michigan, but it has been reported from various places in the southern section of the State.

Daucus Carota L. f. ROSEUS n. f. Flowers pale rose. River Rouge, July 21, 1916, No. 4339; Harris, July 13, 1918, No. 5078.

POLEMONIACEAE.

Phlox divaricata L. f. PURPUREA n. f. Flowers reddish purple. Redford, May 24, 1918, No. 4853; Ypsilanti, May 19, 1918, No. 4839.

P. divaricata L. f. ALBIFLORA n. f. Flowers white. Ypsilanti, May 19, 1918, No. 4838.

VERBENACEAE.

Verbena hastata L. var. *oblongifolia* Nutt. Briefly characterized as with the foliage and flowers of *V. hastata* L., but with the elongated loosely panicled spikes and scattered flowers of *V. urticaefolia* L. Franklin, Sept. 22, 1918, No. 5159, Billington, Farwell, and Gladewitz.

RINGENTACEAE.

VERONICASTRUM VIRGINICUM f. PURPUREUM n. f. (*Leptandra Virginiaca* var. *purpurea* Ph. in Eat & Wright N. Amer. Bot., 297, 1840). Bloomfield, Sept. 8, 1918, No. 5113.

V. Virginicum var. *lanceolatum* f. ROSEUM n. f. Flowers pink or rose colored. Parkedale, July 4, 1918, No. 5027, Billington and Farwell.

PLANTAGINACEAE.

Plantago media L. Cranbrook, 1918, Billington.

SCABIOSACEAE.

Dipsacus Fullonum L. (*D. sylvertris* Huds.) f. TERNATUS n. f. Leaves in threes. Detroit, June 29, 1918, No. 5003.

COMPOSITACEAE.

Aster laevis L. One of our commonest as well as one of the most elegant of asters and very variable. In the type, the plant is often 3 or 4 feet in height, with a loose, open, oblong panicle that is often more than ½ the length of the plant. Stem leaves

clasping by an auriculate base, the lowermost tapering into a winged petiole, the others sessile; the median lanceolate, often 4 or 5 inches long by 1 inch wide, the small subulate leaves of the branches and branchlets strongly auriculate and abruptly contracted above the auricles, thence tapering into an acumination. Near Rochester, Oct. 10, 1918, No. 5205.

Aster laevis var. *laevigatus* (Hooker) A. Gr. Somewhat similar, stem leave often broadly lanceolate (1½-2 inches wide) and thinner, the small leaves of the branches and branchlets subcordate and oblong-lanceolate, not contracted above the base but usually nearly uniform in width for most of their lengths; panicle often shorter an l more ovate. Oxford, Oct. 11, 1917, No. 4721a; Rochester, Oct. 10, 1918, No. 5207.

Aster laevis var. FALCATUS n. var. Panicle usually shorter and ovate; median stem leaves usually broadest at the auriculate base, linear—or oblong—lanceloate, under ¾ inch wide and often 6 inches long, some of them falcate; small subulate leaves as in the preceding variety. Rochester, Oct. 10, 1918, No. 5206.

Aster laevis var. PANDURATUS n. var. Panicle and subulate leaves as in the preceding varieties. Median stem leaves small, 2 or 3 inches long by ½ to 1 inch wide, ovate or oblong, usually pandurate. Rochester, Oct. 10, 1918, No. 5008.

Aster laevis var. *amplifolius* Porter. A not unusual form with the leaves broadly ovate. Rochester, Oct. 10, 1918, No. 5209.

Aster laevis var. THYRSOIDEUS n. var. Similar to the var. *falcatus*, but the inflorescence reduced to a thyrse often a foot or more long. Rochester, Oct. 10, 1918, No. 5209½.

Xanthium glanduliferum Greene. The yellow burrs give it a peculiar appearance at once distinguishing it from the other species. Oakwood, Sept. 15, 1918, No. 5118; River Rouge, Sept. 15, 1918, No. 5139.

Helianthus petolaris Nutt. Scarce. Oakwood, Sept. 15, 1918. No. 5121.

Artemisia gnaphalodes Nutt. Harris. July 13, 1912, No. 5082, Billington & Farwell.

Erechtites hieracifolia var. *prealta* (Raf.) Fernald. Occasional. Detroit, Sept. 20, 1918, No. 5153½.

Senecio obovatus var. *rotundus* Britton. Huron River valley at Ypsilanti, May 19, 1918, No. 4828.

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 264, 1920.

(Reprint from *The Journal of Laboratory and Clinical Medicine*, St. Louis, Vol. VI, No. 1, October, 1920.)

PREPARATION AND STANDARDIZATION OF POLYVALENT ANTIPNEUMOCOCCIC SERUM.*

By N. S. Ferry and Emily Blanchard, Detroit, Mich.

(From the Medical Research Laboratories, Parke, Davis & Co., Detroit, Mich.)

At the present time the requirements of the Hygienic Laboratory, Washington, for the standardization of a polyvalent antipneumococcic serum, call for a serum that shall protect white mice against Type I pneumococcus only. In other words, although the horses which are producing the serum are required to be injected with antigens composed of all types of the organism, the relative strength of the antibodies against these other types is not taken into consideration when the final test of the serum is carried out. Nothing is known, therefore, of the value of this sort of a serum except for Type I pneumonia, and its protective value against the other types is questionable. It is no doubt felt, as the reports of Cole and his associates of the Rockefeller Hospital were unfavorable concerning the protection afforded by Types II and III serum, that to require a standard except for Type I would be without significance and valueless.

However, irrespective of these adverse publications, there is a growing and a healthy demand for a polyvalent antipneumococcic serum from sources that must bear recognition. Many physicians find it impractical and even out of the question to submit cases of pneumonia to a test for proper diagnosis as to the type of the organism responsible for the infection, and are not only willing but anxious to use a polyvalent

*Read before the Society of American Bacteriologists, Boston, Mass., Dec. 29, 1919.

serum. Also, to require a case to wait from eight to twenty-four hours for this diagnosis greatly diminishes the chances of the patient's recovery, and it ought not to be necessary to take this chance.

In fulfilling the requirements for this class of sera, an attempt should be made to produce a serum as potent as possible and one that shall protect mice against all types of the organism in high dilutions, so that some sort of a standard can be adhered to. This is not only a protection for the physician as well as the patient, but, also, a means of checking the results following the administration of the serum, for statistical purposes. At the present time, the reports as regards the clinical value of Types II and III sera are entirely too meagre, and there has been no report following the use of a polyvalent serum standardized against all types. In fact, relatively little has been done or at least published concerning the preparation and standardization of sera except for Type I.

According to Dochez and Avery, the groups of the pneumococcus vary in their pathogenicity for human beings, and they give the order of their virulence as follows: Group III, Group II, Group I, and Group IV; Group III being the most virulent. They state, also, that the degree of protective power developed in the sera of animals immunized against members of the different groups varies inversely with the virulence and with the amount of capsular development. By using 0.5 c.c. of serum and 0.1 c.c. of a bacterial suspension, Cole reported, of Type I serum, a protection against one hundred thousand fatal doses, and, of Type II serum, Avery reported a protection against ten thousand fatal doses. The figures are not given as regards Type III serum, although it was said to be less than Type II, and nothing has been published as regards a polyvalent serum.

With horses immunized against single groups or types, the authors have been able to produce sera of the following strengths: Type I, a protection against ten million M.F.D.; Type II, a protection against ten thousand M.F.D.; Type III, a protection against ten million M.F.D.; and of those

strains of Type IV used, a protection against ten million M.F.D. This does not quite agree with the results of Cole, as his protection against Type III was extremely low.

With individual horses immunized against all types the authors have produced polyvalent antipneumococcic sera showing a uniform protection against all types as follows: Type I, a protection against ten million M.F.D.; Type II, a protection against one hundred thousand M.F.D.; Type III, ten million M.F.D.; and of those strains of Type IV used, a protection against ten million M.F.D.

As far as laboratory animals are concerned, therefore, a sufficient protection with a polyvalent serum was obtained by the authors against Types I, II and III, and for those strains of Type IV which were used as antigens in immunizing the horses, and the serums producing this protection have been standardized against all types, using the method required at the present time by the Hygienic Laboratory for Type I serum.

In producing this polyvalent serum, horses already giving a high titre of Type I serum were chosen and then injected with mixed antigens composed of Types II, III, and IV, using the regular schedule of injections as recommended for Type I. In carrying on this work twelve horses were under treatment. The following chart gives the results of the tests of the sera of the various horses.

STANDARDIZATION OF POLYVALENT ANTIPNEUMOCOCCIC SERA.

Figures represent number of minimum fatal doses of the pneumococcus against which 0.2 c.c. of serum will protect; white mice being used for test animal, as required by the government.

At the present time it is required that 0.2 c.c. of Type I serum will protect against 10,000,000 minimum fatal doses.

ANTIGENS.

SERA.	TYPE I.	TYPE II.	TYPE III.	TYPE IV.
Type I				
Horse 1201	10,000,000		1,000,000	
" 1184	10,000,000		1,000,000	
Type II				
Horse 1203		10,000	1	
" 1204		1,000		
Type III				
Horse 1205			10,000,000	
" 1206			10,000,000	
Type IV				
Horse 1211				10,000,000
" 1212				10,000,000
Polyvalent				
Horse 285	10,000,000	10,000	10,000,000	
" 385	10,000,000	100,000	10 000,000	10,000,000
" 859	10,000,000	100,000	10,000,000	
" 909	10,000,000	10,000	10,000,000	

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Dochez and Avery: Jour. Exper. Med., 1915, xxi. 114.
Avery: Jour. Exp. Med., 1915, xxii. 804.

Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 265, 1920.

(Reprinted from *The Journal of Laboratory and Clinical Medicine*, St. Louis, Vol. VI, No. 2, November, 1920.)

A SIMPLE METHOD OF ISOLATING BACTERIA FROM PATHOLOGIC MATERIAL.

H. C. Klix, Detroit, Mich.

(From the Medical Research Laboratories, Parke, Davis & Co., Detroit, Mich.)

The object of this paper is to present a simple and rapid method for the isolation of bacteria from pathologic material. It has been used by the writer for a number of years in the routine examination of mixed cultures, pus, sputum, and other material submitted for the preparation of autogenous vaccines. It offers a number of advantages over the ordinary plating method.

The use of Petri dishes is not only cumbersome, but is unadapted to certain culture media, particularly those containing unheated body fluids. The exposure incident to handling plates also increases the danger of contamination. The following method overcomes these disadvantages, is simple of execution, and can be satisfactorily applied to practically all bacteriologic examinations.

EQUIPMENT.

A few tubes of suitable culture media, usually ascites agar slants, a small tube of bouillon, and a platinum loop.

TECHNIC.

One-fourth c.c. of bouillon or water of condensation is pipetted into each of four tubes of solid media and the tubes numbered I, II, III, and IV. A small amount of pathologic material is transferred to Tube I and mixed by means of platinum wire with liquid (bouillon or water of condensation). Two or three loopfuls of the bouillon in Tube I are transferred to Tube II and mixed as before. Two loopfuls of bouil-

lon from Tube II are transferred to Tube III. One loopful from Tube III is transferred to Tube IV.

Tubes are shaken to break up any clumps of original material or bacteria, and bouillon is allowed to run over the entire surface of the slant by properly inclining the tube. Tubes are placed in rack in upright position and transferred to incubator. After 24 hours' incubation isolated colonies are picked up by means of platinum wire and transferred to individual tubes of culture media.

Somewhere in the series nicely isolated colonies will be found which can be readily picked up.

The chief advantage of this method aside from its simplicity is its adaptability to any kind of culture media.

**Studies from the Medical Research Laboratories,
Parke, Davis & Co.
Reprint No. 266, 1920.**

(Reprinted from the *Journal of Industrial and Engineering Chemistry*, Vol. 12, No. 12, page 1186, December, 1920.)

THE CHEMISTRY OF DIGITALIS.[1]

By Herbert C. Hamilton.

(From the Medical Research Laboratories, Parke, Davis & Co., Detroit, Mich.)

Few, if any, plants used in medicine have been the subject of such exhaustive research to discover the active principles and separate them in pure form as has digitalis. The list of investigators is seemingly endless; and the products resulting from their researches are equally numerous. While mach of the recent work has been carried out primarily to obtain purified preparations adapted to certain particular purposes or modes of administration, with no specific attempt to isolate a single pure principle or to separate one from another if more than one are present, unquestionably the underlying thought is to accomplish this end.

This is shown by the products, such as digitalone, digipuratum, digipoten, and others, in which the resulting product is more or less purified and is probably better adapted to prompt therapeutic response than the average pharmaceutical.

The author has now succeeded in isolating from digitalis two active agents, although probably not in absolutely pure form. One of these is soluble in chloroform and the other insoluble, but solubility in this reagent is so dependent on the presence or absence of other constituents of digitalis or on a mixture of the two active agents that it is exceedingly difficult to arrive at a point where the complete solubility of the one or insolubility of the other can be established.

If an attempt is made to extract digitalis leaves with chloroform, no considerable amount of active matter is obtained in the extract. If one attempts even to use this as a solvent for Extract Digitalis, U. S. P., little of the active substance is dissolved. On the other hand, a mixture of alcohol and chloroform can be applied with greater success, and under certain

[1]Presented before the Division of Biological Chemistry at the 60th meeting of the American Chemical Society, Chicago, Ill., September 6 to 10, 1920

conditions the whole of the active ingredients will be found in this mixture of solvents.

Again after a partial purification of a digitalis extract, by which the water-soluble inert substances have been removed, chloroform will dissolve all the active material along with the chlorophyll, both of the distinctly different constituents dissolving with equal facility.

It appears, therefore, that certain constituents prevent solution of either active agent in chloroform, while others aid solution, even permitting the ordinarily insoluble principle to dissolve.

By careful manipulation, however, removing first one and then another of the inactive constituents of the leaf, it is possible to arrive at a point where the product is sufficiently free from substances which affect its physical properties to permit the separation from each other of one substance soluble, the other insoluble, in chloroform. Both are soluble in ethyl alcohol, neither entirely soluble in acetone, but this does not seem to indicate the presence of a mixture, the behavior toward acetone being so far unexplainable. Neither is more than slightly soluble in water alone, but the alcoholic solution of the chloroform-insoluble part is clearly soluble in water, even when only a minute amount of alcohol is present, while the chloroform-soluble part from an alcoholic solution gives a hazy solution when mixed with water.

The proportion of the two present in the leaf has not been determined with certainty, since variations are probably chargeable largely to non-quantitative separation. The average ratio, however, is approximately 2 parts chloroform-soluble to 3 parts chloroform-insoluble.

The similarity of the pharmacologic effects of the two substances tends toward skepticism as to the correctness of this separation into two parts of dissimilar solubilities. The existence of the chloroform-insoluble part is unquestionable; but there seems to be a tendency for the soluble part to become less soluble on repeated applications of the solvent, or else conditions tend to favor the separation of more of the insoluble from the soluble. While such tendency toward increasing insolubility is a fact, the writer has never yet suc-

ceeded in obtaining an *inactive* chloroform-soluble portion. In all the work, pharmacologic experiments have demonstrated the presence or absence of an active agent at every stage of the process.

The activities of these two agents, in terms of the leaf of average potency considered as 1, are chloroform-insoluble 100, chloroform-soluble 70. It is evident, therefore, that the chloroform-insoluble principle is responsible for between 65 and 75 per cent of the potency of the leaf, as measured by the M.L.D. or M.S.D. on frogs.

Statements to the effect that the chloroform-soluble part is better adapted to therapeutic purposes than is the insoluble portion must be regarded with suspicion, first, because there appears to be no essential difference in the action of the two, and, second, because the complete separation of the two can be accomplished, if at all, only when in a high state of purity and where no trace of admixture of alcohol appears with the chloroform. At a certain stage in the process, by use of chloroform on a hydro-alcoholic solution of digitalis, one can obtain a separation, one part containing a large percentage of the active agents, the other containing only a small part, but this is merely an incomplete separation of the active from the inactive portion of the extract, mixed miscible solvents and not immiscible solvents being responsible for the completeness of the separation.

The object of presenting this subject in so unfinished a state is primarily to record what seems to be a material bit of progress in the chemistry of digitalis, and at the same time to question publicly any statement to the effect that it is a simple matter to separate the chloroform-soluble constituents from those insoluble in chloroform, and to prove the latter less well adapted to therapeutic use.

It is hoped to present soon a definite method for obtaining one or more active principles of digitalis, together with their chemical and pharmacologic properties. In particular, however, the writer's hope is to find a method by which the active agents can be separated and prepared for therapeutic use in pure form, unmixed with substances which interfere with their beneficial effects and with those which impair their stability.